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(54) Title: CANCER ASSOCIATED PLEXINB1 MUTATIONS

(57) Abstract: The present inventors have identified cancer associated mutations in plexinB1, which is a transmembrane receptor that mediates semaphorin signalling. The frequency of plexinB1 mutations, in particular in prostate and breast cancer, indicates that plexinB1 may be useful in the screening and diagnosis of cancer and as a drug target in the development of anti-cancer therapeutics.

Cancer Associated Plexin B1 Mutations

This invention relates to the identification of cancer-associated mutations in components of the semaphorin signalling pathway, in particular in the plexinB1 transmembrane receptor.

Semaphorins were first identified as extra-cellular cues for axon guidance in the nervous system and have since been shown to have roles in cell-cell communication and cell migration in a variety of adult and embryonic tissues (Kolodkin, A. L. et al. Cell 75 7 (1993): 1389-99; Kolodkin, A. L. Prog. Brain Res. 117 (1998): 115-32; Zou, Y. et al. Cell 102 3 (2000): 363-75).

Semaphorin signalling is mediated by transmembrane receptors called plexins, which can be grouped into 4 sub-families, plexin-A, B, C and D (Tamagnone, L. and P. M. Comoglio. Trends Cell Biol. 10 9 (2000): 377-83; Tamagnone, L. et al. Cell 99 1 (1999): 71-80: WO01/14420).

PlexinB1 has been shown to interact with a variety of factors, including semaphorin 4D, c-Met, neuropilins, active Rac1 and the guanine nucleotide exchange factors (GEFs), PDZ-RhoGEF and LARG (Kolodkin, A. L. et al. Cell 90.4 (1997): 753-62; Hirotsani, M. et al. Biochem. Biophys. Res. Commun. 297.1 (2002): 32-37; Vikis, H. G. et al. Proc. Natl. Acad. Sci. U.S.A 97 23 (2000) 12457-62; Driessens, M. H. et al. Curr.Biol. 11.5 (2001) 339-44; Driessens, M. et al. FEBS Lett. 529.2-3 (2002) 168; Aurandt, J. et al. Proc. Natl. Acad. Sci. U.S.A 99 19 (2002) 12085-90; Perrot, V. et al J.Biol.Chem. 277.45 (2002) 43115-20; Swiercz, J. M. et al. Neuron 35.1 (2002): 51-63). Despite these interactions, the exact function of plexinB1 is not yet known.

The present inventors have now identified mutations in plexinB1 that are closely associated with cancer. The frequency of these mutations, in particular in prostate and breast cancer, indicates that they may be useful in screening and diagnosis of cancer and as drug targets in the development of anti-cancer therapeutics.

One aspect of the invention provides a method of assessing an individual for a cancer condition comprising;
providing a tissue sample obtained from said individual, and;
determining the presence in said sample of one or more cells comprising a plexinB1 nucleic acid sequence having one or more mutations therein.

The presence of one or more cells comprising a mutant plexinB1 nucleic acid sequence in a sample obtained from an individual may be indicative of said individual having or being at risk of having a cancer condition. The one or more mutations in the nucleic acid sequence may alter the expression and/or activity of the encoded plexinB1 polypeptide.

A cancer condition as described herein may include any type of solid cancer and malignant lymphoma and especially leukaemia, sarcomas, skin cancer, bladder cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, oesophageal cancer, pancreas cancer, renal cancer, stomach cancer and cerebral cancer. In particular, methods of the invention may be useful in the treatment of breast or prostate cancer.

An individual may be healthy or may be suspected of or at risk of suffering from a cancer condition. In other embodiments, an individual may be suffering from a cancer condition. In some embodiments, an individual may be undergoing a cancer treatment and methods of the invention may be useful in determining the progress of the treatment.

A sample obtained from an individual may be a tissue sample comprising one or more cells, for example a biopsy from a cancerous tissue as described above, or, in certain contexts, a non-cancerous tissue.

Methods of the invention may be useful in characterising a cancer condition, for example for prognostic purposes. For example, the presence of a plexinB1 nucleic acid or polypeptide having one or more mutations as described herein may indicate that the cancer condition is an invasive or metastatic cancer condition e.g. a cancer condition which is prone to or has a high probability of metastasis. In some embodiments, a method may comprise determining the level or amount of cells having a plexinB1 mutation in sample, for example a sample from a primary tumour. An increased level of cells in the sample comprising a mutant plexinB1 sequence may be indicative that the cancer condition is invasive or susceptible to metastasis.

A mutant plexinB1 nucleic acid may comprise a nucleotide sequence which has one or more mutations relative to the wild-type plexinB1 nucleotide sequence, as set out in AB007867. The mutations may be deletions, insertions or substitutions of one or more nucleotides. The one or more

mutations may be in a coding or non-coding region of the plexin nucleic acid sequence and may alter the expression or function of the plexinB1 polypeptide. In other words, the mutant nucleic acid may encode a mutant plexinB1 polypeptide sequence with aberrant activity, or may encode a wild-type plexinB1 polypeptide which is expressed at an aberrant e.g. an increased or reduced, level, for example by means of an alteration in the activity of a plexinB1 regulatory element. A mutant nucleic acid may have one, two, three, four or more mutations relative to the wild-type sequence.

In some embodiments, the one or more mutations may occur in the region of the nucleic acid which encodes the cytoplasmic domain of the plexinB1 polypeptide, for example in the nucleotide sequence corresponding to exons 22, 23, 24, 25, 26, 27, 28 or 29. In some embodiments, a mutation may be in the Rac1 binding region (i.e. amino acid residues 1724-1870: Driessens et al (2001) Curr Biol 11 339-344). Such a nucleic acid sequence may have a mutation at one or more mutation positions selected from the group consisting of 5059, 5060, 5074, 5107, 5359, 5401, 5452, 5458, 5468, 5474, 5596, 5653, 5662, 5674, 5713, 5714 and 5980 relative to the wild-type plexinB1 coding sequence. The mutation may be selected from the group consisting of C5059T, C5060T, G5074A, A5107G, A5359G, T5401A, G5452A, G5458A, T5468C, A5474G, A5596G, A5653G, C5662T, A5674G, C5713T, T5714C and C5980T:

The nucleic acid sequence may be a genomic sequence, for example a genomic sequence comprising one or more of exons 22, 23, 24, 25, 26, 27, 28 and 29, or may be an RNA or cDNA sequence.

As described above, a mutant plexin nucleic acid sequence may encode a plexinB1 polypeptide having one or more mutations therein. A method of assessing an individual for a cancer condition according to the invention may thus comprise; providing a tissue sample obtained from the individual, and; determining the presence in the sample of one or more cells comprising a mutant plexinB1 polypeptide. The presence of one or more such cells may be indicative of said individual having or being at risk of having a cancer condition.

A mutant plexinB1 polypeptide may comprise an amino acid sequence which has one or more mutations relative to the wild-type plexinB1 sequence set out in AB007867. The mutations may be deletions, insertions or substitutions of one or more amino acids. In some embodiments, the mutations may occur in the cytoplasmic domain of the plexinB1 polypeptide. A mutant polypeptide may have one, two, three, four or more mutations relative to the wild-type sequence.

The one or more mutations may, for example, occur at a mutation site in the plexinB1 amino acid sequence selected from the group consisting of K1613, T1697, G1728, A1730, T1733, T1776, T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735 and R1904. Suitable mutations include substitutions selected from the group consisting of T1697A, G1728S, A1730, T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S, F1711I, G1602T, L1815P, L1815F, K1613E, N1735S and R1904W.

Amino acid residues in the plexinB1 sequence are numbered herein in a N to C direction, starting at the initiating Met residue, which is numbered 1, as set out in AB007867.1 Nucleotide residues are numbered 5' to 3' from the A of the
5 initiating ATG codon, as set out in AB007867.1.

The presence of the one or more cells in the test sample may be determined by detecting the presence of a nucleic acid sequence encoding the mutant plexinB1 polypeptide or
10 by detecting the presence of the mutant plexinB1 polypeptide.

Various methods are available for determining the presence or absence in a test sample of a particular nucleic acid
15 sequence, for example a nucleic acid sequence which has a particular nucleotide at a site of mutation. Furthermore, having sequenced nucleic acid of an individual or sample, the sequence information can be retained and subsequently searched without recourse to the original nucleic acid
20 itself. Thus, for example scanning a database of sequence information using computer or other electronic technology may identify a sequence alteration or mutation.

Methods according to some aspects of the present invention
25 may comprise determining the binding of an oligonucleotide probe to nucleic acid obtained from the sample, for example, genomic DNA, RNA or cDNA. The probe may comprise a nucleotide sequence which binds specifically to a nucleic acid in the presence of one or more mutations and does not
30 bind specifically to the nucleic acid in the absence of the one or more mutations or vice versa.

The oligonucleotide probe may comprise a label and binding of the probe may be determined by detecting the presence of the label.

- 5 A method may include hybridisation of one or more (e.g. two) oligonucleotide probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as
10 part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation.

- Binding of a probe to target nucleic acid (e.g. DNA) may be
15 measured using any of a variety of techniques at the disposal of those skilled in the art. For instance, probes may be radioactively, fluorescently or enzymatically labelled. Other methods not employing labelling of probe include examination of restriction fragment length
20 polymorphisms, amplification using PCR, RN'ase cleavage and allele specific oligonucleotide probing. Probing may employ the standard Southern blotting technique. For instance, DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may
25 then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined.

- 30 Nucleic acid may be screened using a variant- or allele-specific probe. Such a probe may correspond in sequence to a region of the plexinB1 gene, or its complement, which contains one or more of the mutations described herein,

which are shown to be associated with cancer. Under suitably stringent conditions, specific hybridisation of such a probe to nucleic acid from a sample is indicative of the presence of the mutation in the test nucleic acid. For
5 efficient screening purposes, more than one probe may be used on the same test sample.

Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective
10 hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on.

Suitable selective hybridisation conditions for
15 oligonucleotides of 17 to 30 bases include hybridisation overnight at 42°C in 6X SSC and washing in 6X SSC at a series of increasing temperatures from 42°C to 65°C.

Other suitable conditions and protocols are described in
20 Molecular Cloning: a Laboratory Manual: 3rd edition, Sambrook & Russell, 2001, Cold Spring Harbor Laboratory Press NY and Current Protocols in Molecular Biology, Ausubel et al. eds. John Wiley & Sons, 1992.

25 Nucleic acid, which may be genomic DNA, RNA or cDNA, or an amplified region thereof, may be sequenced to identify or determine the presence of a mutation therein. A mutation may be identified by comparing the sequence obtained with the nucleotide sequence of AB007867. A mutation may be a
30 deletion, substitution or insertion of one or more nucleotides relative to the AB007867 sequence. In particular, the presence of one or more mutations

identified herein to be associated with cancer may be determined.

Sequencing may be performed using any one of a range of
5 standard techniques. Sequencing of an amplified product
may, for example, involve precipitation with isopropanol,
resuspension and sequencing using a TaqFS+ Dye terminator
sequencing kit. Extension products may be electrophoresed
on an ABI 377 DNA sequencer and data analysed using
10 Sequence Navigator software.

Since it will not generally be time- or labour-efficient to
sequence all nucleic acid in a sample or even the whole
PlexinB1 gene or coding sequence, a specific amplification
15 reaction such as PCR using one or more pairs of primers may
conveniently be employed to amplify the region of interest
within the nucleic acid sequence, for example, the portion
of the sequence suspected of containing cancer associated
mutations. The amplified nucleic acid may then be
20 sequenced as above, and/or tested in any other way to
determine the presence or absence of a particular feature.

Suitable amplification reactions include the polymerase
chain reaction (PCR) (reviewed for instance in "PCR
25 protocols; A Guide to Methods and Applications", Eds. Innis
et al, 1990, Academic Press, New York, Mullis et al, Cold
Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich
(ed), PCR technology, Stockton Press, NY, 1989, and Ehrlich
et al, Science, 252:1643-1650, (1991)). PCR comprises
30 steps of denaturation of template nucleic acid (if double-
stranded), annealing of primer to target, and
polymerisation. The nucleic acid used as template in the
amplification reaction may be genomic DNA.

Other specific nucleic acid amplification techniques include strand displacement activation, the Q β replicase system, the repair chain reaction, the ligase chain
5 reaction, rolling circle amplification and ligation activated transcription. For convenience, and because it is generally preferred, the term PCR is used herein in contexts where other nucleic acid amplification techniques may be applied by those skilled in the art. Unless the
10 context requires otherwise, reference to PCR should be taken to cover use of any suitable nucleic amplification reaction available in the art.

Methods of the present invention may therefore comprise
15 amplifying a portion of the plexinB1 coding sequence containing one or more sites of mutation from one or more cells from said tissue sample.

Mutation- or variant-specific oligonucleotides may be used
20 in PCR to specifically amplify particular sequences if present in a test sample. Assessment of whether a PCR band contains a gene variant may be carried out in a number of ways familiar to those skilled in the art. The PCR product may for instance be treated in a way that enables one to
25 display the polymorphism on a denaturing polyacrylamide DNA sequencing gel, with specific bands that are linked to the gene variants being selected.

An oligonucleotide for use in nucleic acid amplification
30 may be about 30 or fewer nucleotides in length (e.g. 18, 21 or 24). Generally specific primers are upwards of 14 nucleotides in length, but need not be more than 18-20.

In some embodiments, the region of nucleic acid sample comprising a position of mutation may be amplified using a pair of oligonucleotide primers, of which the first member of the pair comprises a nucleotide sequence which
5 hybridises to a complementary sequence which is proximal to and 5' of the position of mutation, and the second member of the primer pair comprises a nucleotide sequence which hybridises to a complementary sequence which is proximal to and 3' of the position of mutation.

10

Mutation sites within the plexin B1 nucleotide sequence are discussed elsewhere herein.

In other embodiments, the first member of the pair of
15 oligonucleotide primers may comprise a nucleotide sequence which hybridises to a complementary sequence which is proximal to and 5' or 3' of the mutation site, and the second member of the pair may comprise a nucleotide sequence which hybridises under stringent conditions to a
20 sequence which includes a particular nucleotide (i.e. A, G, T or C) at the mutation site and not to sequences which include other nucleotides at the mutation site, such that amplification only occurs in the presence of the particular nucleotide at the mutation site.

25

Another aspect of the invention provides a pair of oligonucleotide amplification primers suitable for use in the methods described herein.

30 A suitable pair of amplification primers according to this aspect may have a first member comprising a nucleotide sequence which hybridises to a complementary sequence which is proximal to and 5' of a mutation site in the plexinB1

coding sequence, for example a mutation site in the sequence encoding the cytoplasmic domain of the polypeptide, such as at position 5059, 5060, 5074, 5107, 5359, 5401, 5452, 5458, 5468, 5474, 5596, 5653, 5662, 5674, 5 5713, 5714 or 5980, and;
a second member comprising a nucleotide sequence which hybridises to a complementary sequence which is proximal to and 3' of the mutation site.

- 10 The first member may hybridise to a first nucleic acid strand having a sequence as shown in AB007867, and the second member may hybridise to a strand complementary to the first strand.
- 15 The identity of the nucleotide at the position of mutation may then be determined by determining the binding of an oligonucleotide probe to the amplified region. A suitable oligonucleotide probe comprises a nucleotide sequence that binds specifically to a sequence comprising a particular
- 20 nucleotide at the mutation site and does not bind specifically to other sequences comprising other residues at the mutation site.

Other suitable pairs of amplification primers may have a

25 first member comprising a nucleotide sequence which hybridises to a complementary sequence which is proximal to and 5' or 3' of a mutation site, for example a mutation site in the sequence encoding the cytoplasmic domain of the polypeptide, such as at position 5059, 5060, 5074, 5107,

30 5359, 5401, 5458, 5452, 5468, 5474, 5596, 5653, 5662, 5674, 5713, 5714 or 5980 of the plexinB1 coding sequence, and a second member of the pair comprising a nucleotide sequence which hybridises under stringent conditions to a sequence

comprising a particular nucleotide at the mutation site and not to sequences having other nucleotides at the mutation site, such that amplification only occurs in the presence of the particular nucleotide.

5

An alternative or supplement to looking for the presence of mutant sequences in a test sample is to look for the presence of the normal, wild type sequence, e.g. using a suitably specific oligonucleotide probe or primer. Use of
10 oligonucleotide probes and primers has been discussed in more detail above.

A further aspect of the present invention provides an oligonucleotide which hybridises specifically to a nucleic
15 acid sequence which comprises a particular nucleotide at a mutation site within the plexinB1 nucleic acid sequence, for example within the coding sequence at a position selected from the group consisting of 5059, 5060, 5074, 5107, 5359, 5401, 5452, 5458, 5468, 5474, 5596, 5653, 5662,
20 5674, 5713, 5714 and 5980.

Such oligonucleotides may be used in a method of screening nucleic acid. Some preferred oligonucleotides have a sequence which is complementary to the plexinB1 coding
25 sequence, or a sequence which differs from such a sequence by addition, substitution, insertion or deletion of one or more nucleotides, but preferably without abolition of ability to hybridise selectively to a sequence comprising a particular residue (i.e. one of A, G, C or T) at a mutation
30 site as described herein, that is wherein the degree of similarity of the oligonucleotide or polynucleotide with one of the sequences given is sufficiently high.

In some preferred embodiments, oligonucleotides according to the present invention are at least about 10 nucleotides in length, more preferably at least about 15 nucleotides in length, more preferably at least about 20 nucleotides in length. Oligonucleotides may be up to about 100 nucleotides in length, more preferably up to about 50 nucleotides in length, more preferably up to about 30 nucleotides in length.

Approaches that rely on hybridisation between a probe and test nucleic acid and subsequent detection of a mismatch may be employed. Under appropriate conditions (temperature, pH etc.), an oligonucleotide probe will hybridise with a sequence that is not entirely complementary. The degree of base pairing between the two molecules will be sufficient for them to anneal despite a mis-match. Various approaches are well known in the art for detecting the presence of a mis-match between two annealing nucleic acid molecules.

For instance, RN'ase A cleaves at the site of a mis-match. Cleavage can be detected by electrophoresing test nucleic acid to which the relevant probe or probe has annealed and looking for smaller molecules (i.e. molecules with higher electrophoretic mobility) than the full-length probe/test hybrid.

Thus, an oligonucleotide probe that has the sequence of a region of the normal plexinB1 coding sequence (either sense or anti-sense strand) in which the mutations associated with cancer or cancer susceptibility as described herein are known to occur, (e.g. the sequence encoding the

cytoplasmic domain) may be annealed to test nucleic acid and the presence or absence of a mis-match determined.

Detection of the presence of a mis-match may indicate the presence in the test nucleic acid of a mutation associated with cancer or cancer susceptibility. On the other hand, an oligonucleotide probe that has the sequence of a region of the gene including a mutation associated with cancer or cancer susceptibility may be annealed to test nucleic acid and the presence or absence of a mis-match determined. The presence of a mis-match may indicate that the nucleic acid in the test sample has the normal sequence (the absence of a mis-match indicating that the test nucleic acid has the mutation). In either case, a battery of probes to different regions of the gene may be employed.

Oligonucleotide probes and primers based on the sequence of plexinB1 may be designed by the ordinary skilled person using conventional primer design software. Those skilled in the art are well versed in the design of suitable primers for use processes such as PCR. Various techniques for synthesizing oligonucleotide primers are well known in the art, including phosphotriester and phosphodiester synthesis methods.

Hybridisation with allele specific oligonucleotides may be conveniently carried out using an oligonucleotide array, preferably a microarray, to determine the identity of a nucleotide present at one or more positions of mutation.

Microarrays are particularly suitable for the detection of polymorphisms (Yershov, G. et. al.(1996) PNAS USA, Genetics, Vol. 93, 4913-4918; Schena, M., 1999, DNA

Microarrays "a practical approach", ISBN, 0-19-963777-6, Oxford press, editor B. D. Hames; Cheung, V. G., et. al., 1999, Nat. Genet., vol. 21, 15-19, WO84/01031, WO88/1058, WO89/01157, WO93/8472, WO95/18376/ WO95/18377, WO95/24649
5 and EP-A-0373203).

In brief, the DNA microarray may be generated using oligonucleotides that have been selected to hybridise with the specific target mutation or polymorphism. These
10 oligonucleotides may be applied by a robot onto a predetermined location of a glass slide, e.g. at predetermined X, Y cartesian coordinates, and immobilised. An amplified genomic product (e.g. fluorescently labelled DNA) is introduced on to the DNA microarray and a
15 hybridisation reaction conducted so that sample RNA or DNA binds to complementary oligonucleotide sequences in a sequence-specific manner, and unbound material is washed away. Amplified sample DNA containing a mutation can thus be detected by its binding to complementary
20 oligonucleotides on the array to produce a signal. The absence of a signal for a specific oligonucleotide probe indicates that the amplified sample does not have the corresponding mutation. The signal produced at each coordinate on the microarray is conveniently read using an
25 automated detector in order to correlate each signal with a particular oligonucleotide.

Nucleic acid, such as an oligonucleotide probe and/or pair of amplification primers, may be provided as part of a kit
30 for performing a method according to the invention, e.g. in a suitable container such as a vial in which the contents are protected from the external environment. The kit may include instructions for use of the nucleic acid, e.g. in

PCR and/or a method for determining the presence of nucleic acid of interest in a test sample. A kit wherein the nucleic acid is intended for use in PCR may include one or more other reagents required for the reaction, such as
5 polymerase, nucleosides, buffer solution etc. The nucleic acid may be labelled. A kit for use in determining the presence or absence of nucleic acid of interest may include one or more articles and/or reagents for performance of the method, such as means for providing or preparing the test
10 sample itself.

Another aspect of the present invention provides a method for determining the presence or absence of a specific nucleotide at a mutation site in the plexinB1 nucleic acid
15 sequence in a test sample comprising:

contacting a plexinB1 nucleic acid sequence with a probe which specifically binds to a mutant plexinB1 nucleotide sequence, for example a nucleotide sequence having a particular residue at a mutation site (i.e. a
20 mutant plexinB1 sequence), such as a sequence encoding a mutant plexinB1 polypeptide as described herein; and,
determining binding of the nucleic acid sequence and the probe.

25 The nucleic acid sequence may comprise one or more mutation sites selected from the group consisting of 5059, 5060, 5074, 5107, 5359, 5401, 5452, 5458, 5468, 5474, 5596, 5653, 5662, 5674, 5713, 5714 and 5980 of the plexinB1 coding sequence. The identity of the nucleotide at the one or more
30 sites of mutation determines the sequence of the mutant plexinB1 nucleic acid and the binding of the probe.

A method for determining the presence or absence in a test sample of a mutation within a test plexinB1 nucleic acid sequence may comprise:

5 determining the identity of the nucleotide at one or more positions of mutation in the test sequence,

the presence of a mutation in the test plexinB1 sequence being inferred by the identity of the nucleotide at the one or more positions of mutation.

10 The nucleotide at the one or more positions of mutation in the test sequence may be compared with the nucleotide at the corresponding one or more positions of mutation in the wild type plexinB1 sequence. The presence of a different nucleotide in the test sequence compared to the wild type
15 sequence at the one or more positions is indicative of the presence of a mutation.

A suitable position of mutation may include a position selected from the group consisting of positions 5059, 5060,
20 5074, 5107, 5359, 5401, 5452, 5458, 5468, 5474, 5596, 5653, 5662, 5674, 5713, 5714 and 5980 of the plexinB1 coding sequence.

A mutation may be a cancer-associated mutation as described
25 herein.

The test sample may be a sample of genomic DNA, cDNA or RNA from a tissue sample obtained from an individual, for example a tumour or other tissue biopsy or a sample of
30 biological fluid, such as a blood sample. The individual may be healthy or may be suffering from a cancer or other condition as described herein.

Optionally, such a method may comprise amplifying the plexinB1 nucleic acid sequence using a pair of oligonucleotide primers. As noted, physical detection may be employed using for example hybridisation of a suitable probe, or a transcription factor or other agent that binds nucleic acid in a sequence-specific fashion, or detection may be performed *in silico* using suitable data analysis techniques, e.g. on a computer.

10 The identity of the nucleotide at a site of mutation may be determined using a method described herein, in particular, the presence of the nucleotide other than the nucleotide in the corresponding wild-type sequence may be determined.

15 Mutations associated with cancer may also be detected at the protein level by detecting the presence of a mutant plexinB1 polypeptide. For practical purposes, or at least commercial purposes bearing in mind cost and time, assessment of target protein expression at the protein level may be preferred over assessment at the nucleic acid level.

A method of determining the presence, absence or level of cancer cells in a sample from an individual, may include contacting a sample with a specific binding member directed against a mutant plexinB1 polypeptide, and determining binding of the specific binding member to the sample. Binding of the specific binding member to the sample may be indicative of the presence of a cancer cell within the sample.

Preferred specific binding molecules for use in aspects of the present invention include antibodies and fragments or

derivatives thereof ('antibody molecules'). Antibody molecules are described in more detail below.

5 A cancer cell may be a cell from any type of cancer, for example as described above. In some preferred embodiments, the cancer cell may be a prostate cancer cell or a breast cancer cell.

A mutant plexinB1 polypeptide may comprise one or more
10 mutations, for example one, two, three or more mutations, relative to the wild-type sequence. In some embodiments, the one or more mutations may occur within the cytoplasmic domain of the polypeptide. Suitable mutations may occur at mutation sites T1697, G1728, A1730, K1613, T1733, T1776,
15 T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735 and R1904 in the plexinB1 amino acid sequence and may, for example, include one or more substitutions selected from the group consisting of T1697A, G1728S, A1730T, K1613E, T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S,
20 F1711I, G1602T, L1815F, L1815P, N1735S and R1904W.

In particular, mutations in plexinB1 nucleic acid and polypeptide sequences are associated with invasive cancers which are prone to metastasise and methods of the invention may be used to determine the presence of invasive cancer
25 cells in a sample obtained from an individual.

Another aspect of the present invention provides for a method of categorising a tissue as (i) normal, (ii) potentially or actually pre-cancerous or cancerous,
30 dysplastic, or neoplastic or (iii) cancerous and prone to metastasise, the method including determining binding to a sample of the tissue of a specific binding member directed against a mutant plexinB1 polypeptide. The pattern or

degree of binding may be compared with that for a known normal sample and/or a known abnormal sample.

A method of the invention may be used to characterise a cell, for example a cancer cell, as invasive or non-invasive, for example in the prognosis of a cancer condition. Thus, binding of (e.g.) an anti-mutant plexinB1 specific binding member to a sample provides for categorising the tissue from which the sample is derived as potentially or actually pre-cancerous or cancerous, dysplastic or neoplastic or cancerous and potentially metastatic. The method may be used to pre-screen samples before further analysis. The method may also be useful for screening or analysis of samples previously tested using another technique.

A specific binding molecule, for example an antibody molecule, may be provided in a kit, which may include instructions for use in accordance with a method of the invention. Such kits are provided as a further aspect of the invention. One or more other reagents may be included, such as labelling molecules, and so on (see below). Reagents may be provided within containers, which protect them from the external environment, such as a sealed vial. A kit may include one or more articles for providing or preparing the test sample itself, depending on the tissue of interest. A kit may include any combination of, or all of, a blocking agent to decrease non-specific staining, a storage buffer for preserving binding molecule activity during storage, staining buffer and/or washing buffer to be used during antibody staining, a positive control, a negative control and so on. Positive and negative controls may be used to validate the activity and correct usage of

reagents employed in accordance with the invention and which may be provided in a kit. Controls may include samples, such as tissue sections, cells fixed on coverslips and so on, known to be either positive or negative for the presence of the mutant plexinB1. The design and use of controls is standard and well within the routine capabilities of those of ordinary skill in the art.

Samples to be subjected to a contact with a binding member in accordance with methods of the invention may be prepared using any available technique which allows binding of a specific binding molecule to the mutant plexinB1 polypeptide. Various techniques are standard in the art.

The reactivities of a binding member such as an antibody on normal and test samples may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding binding molecule (e.g. antibody) and reporter molecule.

One favoured mode is by covalent linkage of each binding member with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine. Other reporters include macromolecular colloidal particles or

particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically
5 detected or otherwise recorded. These molecules may be enzymes that catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in
10 characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed. Further examples are horseradish peroxidase and
15 chemiluminescence.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and
20 general knowledge.

Methods of assessing a cancer condition as described herein may be employed before, during and/or after a course of cancer treatment in order to determine the progress and/or
25 effectiveness of the treatment.

A further aspect of the invention provides an antibody molecule that binds specifically to mutant plexinB1 polypeptide. Such an antibody binds preferentially to
30 mutant plexinB1 polypeptide relative to wild-type plexinB1 polypeptide.

Antibody molecules may be useful both in the diagnosis and therapy of cancer, in accordance with the invention.

Antibodies that are specific for a mutant plexinB1 polypeptide may be obtained using techniques that are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep, monkey or bird such as chicken) with the mutant protein or a fragment thereof, or a cell or virus that expresses the protein or fragment.

Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to mutant plexinB1, or fragments comprising the cytoplasmic domain thereof. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, Nature 357: 80-82).

The production of specific monoclonal antibodies is also well established in the art.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a target may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with the target or may be one constructed using sequences obtained from an

organism which has been exposed to the antigen of interest (or a fragment thereof).

An antibody molecule which is specific for a mutant plexinB1 may be conjugated or bound to a cytotoxic agent. Binding of the antibody molecule to the mutant plexinB1 may be used to selectively target the cytotoxic agent to a cancer cell. Many suitable cytotoxic agents are known in the art.

10

Another aspect of the invention provides a method of identifying and/or obtaining an antibody specific for a mutant plexinB1, the method comprising;

15 providing a population of antibody molecules specific for mutant plexinB1,

contacting said population with a normal plexinB1 polypeptide,

20 identifying one or more members of said population which bind preferentially to mutant plexinB1 relative to normal plexinB1

A method may include isolating and/or purifying said one or more members. A population of antibody molecules specific for mutant plexinB1 may be obtained as described above.

25

Antibodies may be modified in a number of ways. Indeed, unless context precludes otherwise, the term "antibody" should be construed as covering any specific binding substance having an antibody antigen-binding domain. Thus, 30 this covers antibody fragments, derivatives, and functional equivalents, including any polypeptide comprising an immunoglobulin binding domain, whether natural or synthetic. Chimaeric molecules comprising an

immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimaeric antibodies are described in EP-A-0120694 and EP-A-0125023.

5

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')₂ fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

15

Recombinant expression of polypeptides, including antibody molecules, is well-known in the art.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is E. coli. The preferred hosts for baculovirus expression are insect cells such as the SF9 cell line.

25

30

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences,

enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 3rd edition, Sambrook & Russell 2001, Cold Spring Harbor Laboratory Press. Transformation procedures suitable for different hosts are well known in the art.

Following production by expression from encoding nucleic acid an antibody or other specific binding molecule directed against mutant or wild-type plexinB1, may be recovered and may be isolated, if necessary conjugated to an appropriate label or reporter, and provided for use in assessing a cancer condition in an individual as described herein.

Determination of binding to mutant plexinB1 polypeptide in vivo may be used to identify localisations of cancer cells in the body. Labelled binding molecules against mutant plexinB1 may be administered to an individual and binding within the body determined. When a radionucleotide such as Iodine-125, Indium-111, Thallium-201 or Technetium-99m is attached to an antibody, and the antibody localises preferentially in tumour rather than normal tissues, the presence of radiolabel in tumour tissue can be detected and quantitated using a gamma camera or scintigraphy.

Radiolabelling with technetium-99m is described in Pak et al (1992), Nucl. Med. Biol. 19; 699-677. A review of cancer imaging with anti-CEA antibodies is provided by Goldenberg D.M., Int. J. of Biol. Markers 1992, 7; 183-188. The present invention of course extends to specific binding members directed against mutant plexinB1 as disclosed, for use in any such in vivo method.

As described above, mutations in the plexinB1 nucleic acid and amino acid sequences have further been shown by the inventors to be associated with the invasiveness of a cancer cell. Thus, methods of the invention may be useful
5 in the prognosis of an individual having a cancer condition.

Another aspect of the invention provides a method of determining the invasiveness of a cancer cell from a sample
10 obtained from an individual, the method comprising,
determining the presence or absence in said cell of a plexinB1 nucleic acid having one or more mutations therein, the presence of said plexinB1 nucleic acid being indicative that the cancer cell is invasive.

15 Mutant plexinB1 nucleic acid is described in detail above. In particular, a mutant plexinB1 nucleic acid from an invasive cancer cell may have two or more, or three or more mutations relative to the wild-type sequence. The cancer
20 cell may be a prostate cancer cell or other cancer cell as described above.

In some embodiments a mutant plexinB1 nucleic acid from an invasive cancer cell may include one or more of the
25 mutations T1795A, P1597L, P1597S and L1815P. In some preferred embodiments, a mutant plexinB1 includes the mutation T1795A.

The presence of a mutant plexinB1 nucleic acid may be
30 determined by detecting the presence of a polypeptide sequence encoded by said mutant plexinB1 polypeptide or by directly detecting the mutant plexinB1 nucleic acid, as described above.

The results set out herein show that the proportion of cells containing plexinB1 mutations is increased in metastatic tumours compared to primary tumours.

5

A method of determining the invasiveness or susceptibility to metastasis of a cancer condition in an individual may comprise;

10 determining the presence, level or amount of cancer cells which comprise a plexinB1 nucleic acid sequence having one or more mutations therein in a sample obtained from said individual.

15 The presence of said cells in the sample or the presence of an elevated level of said cells relative to cells with wild-type plexinB1 may be indicative that the cancer condition is susceptible to or at risk of metastasis.

20 Another aspect of the invention provides a method of identifying and/or obtaining a compound as a putative anti-cancer agent, the method comprising;

contacting a plexinB1 polypeptide with a test compound, and;

25 determining the activity of the plexinB1 polypeptide in the presence relative to the absence of test compound.

A decrease in activity in the presence relative to the absence of test compound is indicative that the compound is a putative anti-cancer agent.

30

The activity of the plexinB1 polypeptide may be determined by determining the binding of said polypeptide to a ligand such as semaphorin 4D, neuropilin, c-Met, active Rac1, PDZ-

RhoGEF or LARG and/or by determining the activation of Rho A or the binding of Rho A to effector proteins (e.g. ROCK).

In some embodiments, the plexinB1 polypeptide may be a mutant plexinB1 polypeptide as described above. In an optional step following the identification of a test compound which alters the activity of the mutant plexinB1 polypeptide, the effect of the test compound on wild-type plexinB1 may be determined. A method according to such
10 embodiments may comprise contacting a wild-type plexinB1 polypeptide with the test compound, and; determining the activity of the wild-type plexinB1 polypeptide.

In some embodiments, a putative anti-cancer agent may
15 disrupt the interaction of a mutant plexinB1 polypeptide with a wild-type polypeptide. A method of the invention may comprise the steps of;

contacting the mutant plexinB1 polypeptide with the test compound in the presence of a wild-type plexinB1, and;
20 determining the activity of the wild-type plexinB1 polypeptide.

A test compound which preferentially inhibits the activity of mutant plexinB1 relative to wild type plexinB1 may be
25 particularly useful in specifically targeting cancer cells.

In other embodiments, the plexinB1 polypeptide may be a wild-type plexinB1 polypeptide. A test compound may be identified which alters the activity of both the wild-type
30 and mutant plexinB1 polypeptide. Such a compound may be particularly useful in treating cancer when specifically targeted to a tumour site, for example using tumour-specific antibodies.

An agent identified using one or more primary screens (e.g. in a cell-free system) as having ability to modulate the activity of plexinB1 may be assessed further using one or
5 more secondary screens. A secondary screen may involve testing for effects on growth of cancer cells in vitro, in particular anchorage independent growth, or a biological function of plexinB1 or semaphorin signalling, for example in an animal model.

10

Suitable biological functions which may be assessed in a secondary screen include reduction in size or number of tumours, inhibition of metastasis, or a reduction in other symptoms or effects of a cancer condition.

15

In some embodiments, the activity of the plexinB1 polypeptide may be determined by measuring the tumourigenicity in an animal model of cancer cells, such as NIH3T3 cells, which express the plexinB1 polypeptide.

20

Suitable animal models include athymic nude mice. A reduction in the rate of tumour production in the presence relative to the absence of test compound may be indicative that the compound is a putative anti-cancer agent which enhances or restores the tumour suppressor activity of

25

plexinB1.

In some embodiments, test compounds may be screened initially using a secondary screen as described above, without an initial primary screening step.

30

One class of putative inhibitor compounds can be derived from the plexinB1 polypeptide, in particular the mutant plexinB1 polypeptide, and/or ligands which bind to it,

- including semaphorin 4D, active Rac1, neuropilin, PDZ-RhoGEF and LARG. Membrane permeable peptide fragments of from 5 to 40 amino acids, for example, from 6 to 10 amino acids may be tested for their ability to disrupt such interaction or activity. In some embodiments, peptide fragments may comprise the cytoplasmic domain of plexinB1, in particular one or more mutation sites within the cytoplasmic domain, as described herein.
- 10 Peptide fragments may also be generated wholly or partly by chemical synthesis. The compounds of the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are
- 15 broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied
- 20 Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if
- 25 desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.
- 30 The inhibitory properties of a peptide fragment as described above may be increased by the addition of one of the following groups to the C terminal: chloromethyl ketone, aldehyde and boronic acid. These groups are

transition state analogues for serine, cysteine and threonine proteases. The N terminus of a peptide fragment may be blocked with carbobenzyl to inhibit aminopeptidases and improve stability (Proteolytic Enzymes 2nd Ed, Edited
5 by R. Beynon and J. Bond, Oxford University Press, 2001).

Another convenient way of producing polypeptide molecules, which may be full-length sequences or peptide fragments thereof, for use in methods of the invention, is to express
10 nucleic acid encoding it, by use of nucleic acid in an expression system.

Antibodies which specifically bind to wild-type or mutant plexinB1 polypeptide, or regions thereof, form a further
15 class of putative inhibitor compounds. Candidate inhibitor antibodies may be characterised and their binding regions determined to provide single chain antibodies and fragments thereof which alter (i.e. enhance or disrupt) the interactions and/or activity of plexinB1.

20 Techniques for obtaining antibodies are standard in the art and are described elsewhere herein.

Antibody molecules may for example be micro-injected into
25 cells, e.g. at a tumour site, subject to radio- and/or chemo-therapy (as discussed already above). Antibodies may be employed in accordance with the present invention for other therapeutic and non-therapeutic purposes; which are discussed elsewhere herein.

30 Other candidate inhibitor compounds may be based on modelling the 3-dimensional structure of a polypeptide or peptide fragment and using rational drug design to provide

potential modulator (for example, inhibitor) compounds with particular molecular shape, size and charge characteristics.

5 A potential modulator compound may be a "functional analogue" of a peptide or other compound which modulates mutant plexinB1 activity. A functional analogue has the same functional activity as the peptide or other compound in question, i.e. it may alter (i.e. enhance or interfere
10 with) the binding between mutant plexinB1 and one or more ligands. Examples of such analogues include chemical compounds which are modelled to resemble the three dimensional structure of the mutant or wild-type plexinB1 polypeptide, and in particular the arrangement of the key
15 amino acid residues as they appear in the mutant form of plexinB1.

Mutant or wild-type plexinB1 polypeptide and binding partners may be used in methods of designing mimetics of
20 these molecules suitable for inhibiting mutant plexinB1 activity.

Accordingly, the present invention provides a method of designing mimetics of wild-type or mutant plexinB1
25 polypeptide having the biological activity of modulating, e.g. enhancing or inhibiting, the activity of wild-type or mutant plexinB1 polypeptide, said method comprising:
(i) analysing a substance having the biological activity to determine the amino acid residues essential and
30 important for the activity to define a pharmacophore; and,
(ii) modelling the pharmacophore to design and/or screen candidate mimetics having the biological activity.

Suitable modelling techniques are known in the art. This includes the design of so-called "mimetics" which involves the study of the functional interactions of the molecules and the design of compounds which contain functional groups arranged in such a manner that they could reproduced those interactions.

The modelling and modification of a 'lead' compound to optimise its properties, including the production of mimetics, is further described below.

As described above, the activity or function of a mutant plexinB1 may be inhibited, as noted, by means of a compound that interferes in some way with the interaction of plexinB1 with other factors described herein. An alternative approach to inhibition employs regulation at the nucleic acid level to inhibit activity or function by down-regulating production of the mutant form of plexinB1.

A method of identifying and/or obtaining a compound which is a putative anti-cancer agent may comprise;
contacting a plexinB1 nucleic acid therein with a test compound, and;
determining the expression of the plexinB1 nucleic acid in the presence relative to the absence of test compound.

PlexinB1 nucleic acid sequences may be mutant plexinB1 sequences i.e. sequences having one or more mutations, or wild-type sequences. PlexinB1 nucleic acid sequences are described elsewhere herein. The plexinB1 nucleic acid may be within a cell, for example in a cancer cell line, such as a breast or prostate cancer cell line.

Expression of the nucleic acid may be determined using conventional techniques, such as Northern blotting or RT-PCR.

- 5 Anti-sense or RNAi technology may be used to inhibit the expression of a plexinB1 nucleic acid, in particular a mutant plexinB1 nucleic acid. The use of these approaches to down-regulate gene expression is now well-established in the art.
- 10 Methods of the present invention may include identifying the test compound as a putative anti-cancer agent. Methods may further include isolating, purifying, synthesising and/or manufacturing a compound identified as a putative anti-cancer agent.
- 15 Optionally, compounds identified as putative anti-cancer agents using a method described herein may be modified to optimise activity or provide other beneficial characteristics such as increased half-life or reduced side
- 20 effects upon administration to an individual.

Methods of the present invention may further include formulating a compound identified as a putative anti-cancer agent into a composition, such as a medicament,

25 pharmaceutical composition or drug, with a pharmaceutically acceptable excipient as described below.

Another aspect of the invention provides a pharmaceutical composition comprising the compound as described above and

30 a pharmaceutically acceptable excipient. Such a composition may be administered to an individual.

A method of making a pharmaceutical composition may comprise,

identifying a compound as modulator of plexinB1, for
5 example an inhibitor of mutant plexin B1 or an enhancer of
wild-type plexin B1, using a method described herein,
synthesising, preparing or isolating said modulator,
admixing the modulator with a pharmaceutically
acceptable excipient, vehicle or carrier, and optionally
10 other ingredients to formulate or produce said composition;
and, optionally, determining the activity of mutant
plexinB1 as described herein in the presence of said
composition.

15 In other embodiments, a method of producing a
pharmaceutical composition may comprise;

identifying a compound which modulates the activity of
a plexinB1 polypeptide using a method described herein;
and,
20 admixing the compound identified thereby with a
pharmaceutically acceptable carrier.

The formulation of compositions with pharmaceutically
acceptable carriers is described further below.

25

Another aspect of the invention provides a method for
preparing a pharmaceutical composition, for example, for
the treatment of a cancer condition comprising;

i) identifying a compound which is an agonist/antagonist of
30 a plexinB1 polypeptide
ii) synthesising the identified compound, and;
iii) incorporating the compound into a pharmaceutical
composition.

As described above, a plexinB1 polypeptide may be a wild-type or mutant plexinB1 polypeptide.

- 5 The identified compound may be synthesised using conventional chemical synthesis methodologies. Methods for the development and optimisation of synthetic routes are well known to a skilled person.
- 10 The compound may be modified and/or optimised as described above.

Incorporating the compound into a pharmaceutical composition may include admixing the synthesised compound
15 with a pharmaceutically acceptable carrier or excipient.

The modification of a known pharmacologically active compound to improve its pharmaceutical properties is a known approach to the development of pharmaceuticals based
20 on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, e.g. peptides are not well suited as active agents for oral compositions as they tend to be quickly
25 degraded by proteases in the alimentary canal. The design, synthesis and testing of modified active compounds, including mimetics, may be used to avoid randomly screening large number of molecules for a target property.

- 30 There are several steps commonly taken in modifying a compound which has a given target property. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are

determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled to according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g. spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process.

In a variant of this approach, the three-dimensional structure of plexinB1, in particular mutant plexinB1 as described herein, and ligands are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the modified compound is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade in vivo, while retaining the biological activity of the lead compound. Modified compounds found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or

modification can then be carried out to arrive at one or more final compounds for in vivo or clinical testing.

Those of skill in the art may vary the precise format of
5 methods of the invention using routine skill and knowledge.

Aspects of the invention provide a compound obtained by a method as described above for use in a method of treatment and the use of such a compound in the manufacture of a
10 medicament for use in the treatment of cancer.

Another aspect of the invention provides a method of treating a cancer condition, such as prostate cancer or breast cancer, in an individual, the method comprising
15 inhibiting the activity of mutant plexinB1 polypeptide in one or more cells of said individual.

The activity or function of mutant plexinB1 polypeptide may be inhibited, for example, by administering an antagonist
20 of mutant plexinB1 to said individual. A suitable antagonist may be an antibody molecule, peptide or small organic molecule, and may be obtained by a method described above.

25 As described above, an antagonist of mutant plexinB1 may be specific for mutant plexinB1 or may have an antagonistic effect on both wild-type and mutant plexinB1. An antagonist which acts on both wild-type and mutant plexinB1 may be specifically targeted to a tumour site using conventional
30 techniques.

An alternative approach to inhibition employs regulation at the nucleic acid level to inhibit activity or function by

down-regulating production of mutant plexinB1. The activity of mutant plexinB1 polypeptide may be inhibited by reducing or abolishing expression of plexin B1 polypeptide using anti-sense or RNAi technology.

- 5 Anti-sense oligonucleotides may be designed to hybridise to the complementary sequence of nucleic acid, pre-mRNA or mature mRNA, interfering with the production of mutant plexinB1 polypeptide so that its expression is reduced or completely or substantially completely prevented. In
10 addition to targeting coding sequence, anti-sense techniques may be used to target control sequences of a gene, e.g. in the 5' flanking sequence, whereby the antisense oligonucleotides can interfere with expression control sequences. The construction of antisense sequences
15 and their use is described for example in Peyman and Ulman, Chemical Reviews, 90:543-584, (1990) and Crooke, Ann. Rev. Pharmacol. Toxicol., 32:329-376, (1992).

- Oligonucleotides may be generated in vitro or ex vivo for administration or anti-sense RNA may be generated in vivo
20 within cells in which down-regulation is desired. Thus, double-stranded DNA may be placed under the control of a promoter in a "reverse orientation" such that transcription of the anti-sense strand of the DNA yields RNA which is complementary to normal mRNA transcribed from the sense
25 strand of the target gene. The complementary anti-sense RNA sequence is thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein. Whether or not this is the actual mode of action is still uncertain. However, it is
30 established fact that the technique works.

The complete sequence corresponding to the coding sequence in reverse orientation need not be used. For example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen
5 fragments of various sizes and from various parts of the coding or flanking sequences of a gene to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG codon, and perhaps one or more nucleotides upstream of the initiating codon.
10 A suitable fragment may have about 14-23 nucleotides, e.g. about 15, 16 or 17.

An alternative to anti-sense is to use a copy of all or part of the target gene inserted in sense, that is the same, orientation as the target gene, to achieve reduction
15 in expression of the target gene by co-suppression; Angell & Baulcombe (1997) The EMBO Journal 16,12:3675-3684; and Voinnet & Baulcombe (1997) Nature 389: pg 553). Double stranded RNA (dsRNA) has been found to be even more effective in gene silencing than both sense or antisense
20 strands alone (Fire A. et al Nature, Vol 391, (1998)). dsRNA mediated silencing is gene specific and is often termed RNA interference (RNAi).

RNA interference is a two-step process. First, dsRNA is cleaved within the cell to yield short interfering RNAs
25 (siRNAs) of about 21-23nt length with 5' terminal phosphate and 3' short overhangs (~2nt). The siRNAs target the corresponding mRNA sequence specifically for destruction (Zamore P.D. Nature Structural Biology, 8, 9, 746-750, (2001))

30 RNAi may be also be efficiently induced using chemically synthesized siRNA duplexes of the same structure with 3'-

overhang ends (Zamore PD et al. Cell, 101, 25-33, (2000)).
Synthetic siRNA duplexes have been shown to specifically
suppress expression of endogenous and heterologous genes
in a wide range of mammalian cell lines (Elbashir SM. et
5 al. Nature, 411, 494-498, (2001))

Another possibility is that nucleic acid is used which on
transcription produces a ribozyme, able to cut nucleic acid
at a specific site - thus also useful in influencing gene
expression. Background references for ribozymes include
10 Kashani-Sabet and Scanlon, 1995, Cancer Gene Therapy, 2(3):
213-223, and Mercola and Cohen, 1995, Cancer Gene Therapy,
2(1), 47-59.

Thus, an inhibitor of mutant plexinB1 activity may comprise
15 a nucleic acid molecule comprising all or part of the
mutant or wild type plexinB1 coding sequence or the
complement thereof

Such a molecule may suppress the expression of the mutant
20 plexinB1 polypeptide and may comprise a sense or anti-sense
mutant plexinB1 coding sequence or may be a mutant plexinB1
specific ribozyme, according to the type of suppression to
be employed.

25 The type of suppression will also determine whether the
molecule is double or single stranded and whether it is RNA
or DNA.

A related aspect of the invention provides a method of
30 treating a cancer condition, such as prostate cancer and
breast cancer, in an individual, the method comprising

increasing the activity of wild-type plexinB1 polypeptide in one or more cells of said individual.

Wildtype plexinB1 activity may be enhanced by increasing
5 the level of plexinB1 polypeptide in the one or more cells, for example by expression from a recombinant plexinB1 coding sequence, or by administration of a plexinB1 agonist molecule.

10 Further aspects of the invention provide a nucleic acid encoding wild-type or mutant plexinB1 polypeptide, its complement or a fragment thereof for use in a method of treatment, for example in a method of treating cancer, for example prostate cancer and the use of nucleic acid
15 encoding wild-type or mutant plexinB1, its complement or a fragment thereof in the manufacture of a medicament for the treatment of cancer. Such molecules may, for example, be useful in suppressing the expression of mutant plexinB1 or increasing the expression of wild-type plexin B1.

20 Mutations in the plexinB1 coding sequence are shown herein to be associated with anchorage-independent growth and metastasis. A method of reducing the invasiveness of a tumour in an individual may comprise inhibiting the
25 activity of mutant plexinB1 polypeptide in one or more cells of said tumour, or enhancing the activity of wild-type plexinB1 polypeptide, as described above.

A compound may be administered in a precursor form, for
30 conversion to an active form by an activating agent produced in, or targeted to, the cells to be treated. This type of approach is sometimes known as ADEPT or VDEPT; the former involving targeting the activating agent to the

cells by conjugation to a cell-specific antibody, while the latter involves producing the activating agent, e.g. an enzyme, in a vector by expression from encoding DNA in a viral vector (see for example, EP-A-415731 and WO
5 90/07936).

Whether it is a polypeptide, peptide, nucleic acid molecule, small molecule or other pharmaceutically useful compound that is to be given to an individual,
10 administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and
15 time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

20

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

25 Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled
30 in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral,

or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be
5 in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil.
10 Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or
15 injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for
20 example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, or Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

25 Aspects of the present invention will now be illustrated with reference to the following experimental exemplification, by way of example and not limitation. Further aspects and embodiments will be apparent to those
30 of ordinary skill in the art. All documents mentioned in this specification are hereby incorporated herein by reference.

Figure 1 shows the expression of plexinB1 in LNCaP, PC3 and DU145 cells using quantitative RT-PCR. Real time RT-PCR was performed on a Taqman 7700 using β actin template as control, average of 3 RNA extractions.

5

Figure 2 shows the number of colonies of $\geq 200 \mu\text{M}$ produced after 3 weeks in 0.3% agarose by cells transfected with plexinB1 (A5359G), plexinB1 (A5653G), plexinB1 (T5714C), wild type plexinB1, empty vector or parental NIH3T3 cells.

10 The bars indicate the mean number of colonies produced by 2 independent clones of each construct.

Figure 3 shows sequencing data for plexin B1 prostate cancer mutations.

15 Figure 3a: C5060T (pro1597Leu): i) normal sequence, ii) SSCP band and iii) direct sequence of tumour sample. Figure 3b: C5059 (Pro1597Ser) SSCP band.

Figure 3c: G5074A (Gly1602Thr) complementary sequence: i) normal sequence, ii) SSCP band and iii) direct sequence of
20 tumour sample.

Figure 3d: A5359G (Thr1697Ala): i) normal sequence and ii) direct sequence of tumour sample.

Figure 3e: T5401A (Phe1711Ile) complementary sequence: i) normal sequence and ii) SSCP band..

25 Figure 3f: C5468T (Thr1733Ile): i) normal sequence, ii) SSCP band and iii) direct sequence of tumour sample.

Figure 3g: A5474G (Asn1735Ser): i) normal sequence and ii) direct sequence of tumour sample.

Figure 3h: A5596G (Thr1774Ala): i) normal sequence and ii)
30 SSCP band

Figure 3i: A5653G (Thr1795Ala): i) normal sequence, ii) SSCP band and iii) direct sequence of tumour sample.

Figure 3j: C5662T (Pro1798Ser): i) normal sequence, ii) SSCP band and iii) direct sequence of tumour sample.

Figure 3k: A5674G (Thr1802Ala): i) normal sequence and ii) SSCP band.

- 5 Figure 3l: T5714C (Leu1815Pro) complementary sequences: i) normal sequence, ii) SSCP band and iii) direct sequence of tumour sample.

Figure 3m: C5980T (Arg1904Trp) complementary sequences: i) normal sequence, ii) SSCP band and iii) direct sequence of
10 tumour sample.

Figure 4 shows the sequencing of PCR products from 7 primary prostate cancers. The arrow marks the position of the A5653G (Thr1795Ala) mutation.

15

Figure 5 shows an alignment of plexinB1 sequences with positions of the mutations shown.

Figure 6 shows the tumour growth rates of NIH3T3 stable
20 transfectants. Cells transfected with plexinB1 (A5359), plexinB1 (A5653G), plexinB1 (T5714C), wild type plexinB1, empty vector, or parental cells were injected subcutaneously. 5 mice were injected on each flank with 10 million cells.

25

Figure 7 shows individual plots of tumour growth rate of NIH3T3 cells transfected with plexinB1 (A5359), plexinB1 (A5653G), plexinB1 (T5714C), wild type plexinB1, empty vector, or parental cells and injected subcutaneously into
30 each flank of 5 mice (maximum 10 separate sites). The increase in volume of each tumour is shown. In some cases, animals had to be killed before the tumour in the opposite flank had started to grow.

Figure 8 shows the sequencing data for the breast cancer mutations.

Figure 8a. T5059C (Pro1597Ser) complementary sequences: i)
5 normal sequence and ii) SSCP band.

Figure 8b. C5060T (Pro1597Leu) complementary sequence: i)
SSCP band.

Figure 8c. G5424A (Gly1728Ser) complementary sequences: i)
normal sequence and ii) SSCP band.

10 Figure 8d. G5458A (Ala1730Thr) complementary sequences: i)
normal sequence and ii) SSCP band.

Figure 8e. C5713T (Leu1815Phe) complementary sequences: i)
normal sequence and ii) SSCP band.

15 Table 1 shows plexinB1 mutations in prostate cancer.

Table 2 shows plexinB1 mutations in breast cancer.

Experimental

Materials and Methods

20 Sample Preparation

Paraffin sections were cut from 95 cases of primary prostate cancer treated by radical prostatectomy and 11 metastatic cancers from patients who had relapsed following hormone treatment. Areas of cancer were identified and
25 microdissected from the sections or obtained by laser microdissection, depending on the size of the cancer foci. PCR products were sequenced directly by cycle sequencing (4pmole primer, Amplitaq, ABI)

In vitro mutagenesis

30 The A5359G, A5653G, and T5714C sequence changes

were introduced using Quickchange kit (Stratagene) with the following primers:

GTCCATCTGTCTGTATGCCTTCGTGAGGGTGAG and
CTCACCCCTCACGAAGGCATACAGACAGATGGAC,

- 5 GGAGTGCCTCTCGCCCAGCGGCCAGACCCTCG and
CGAGGGTCTGGCCGCTGGGCGAGAGGCACTCC,
GGTGGCCGGGCACCCCATTTCTTTCTGACGAGG and
CCTCGTCAGAAAGAATGGGGTGCCCGGCCACC.

- The sequence change was confirmed by sequencing. No other
10 sequence change was seen in the cytoplasmic domain.

Transfection

- NIH3T3 cells were transfected with pcDNA3-PlexB1(A5359G),
pcDNA3-PlexB1(A5653G), pcDNA3-PlexB1(T5714C), pcDNA-PlexB1
and pcDNA3 (vector only) by calcium phosphate transfection
15 and selected with G418.

Cell culture

LNCaP, PC3 and DU145 were grown in RPMI-1640 with 10%FCS,
NIH3T3 was grown in DMEM with 10%NCS.

Anchorage independent assays

- 20 1000 cells were grown in 12 well plates in 0.35% agarose
over a layer of 0.7% agarose in medium.

In vivo tumourigenesis assays

- NIH3T3 transfected with mutant, wild type or empty vector
or parental NIH3T3 cells were injected subcutaneously into
25 athymic nude mice.

Screening for Polymorphisms

Control DNA from unrelated individuals of
Caucasian origin was screened for the presence of
identified mutations by

PCR and restriction enzyme digestion (NEB) using HaeIII (P1597L, P1597S, A1730T), AvaII (P1597S), Styl (G1602T), BstZ171 (T1697A), BclI (F1711I), BstNI (G1728S), Styl (T1733I), BsrBI (N1735S), HgaI (T1776A), HphI (T1795A),
5 HaeIII (P1798S), HhaI (T1802A), MnlI (L1815P, L1815F) and TaqI (R1904W). Primers with a single base pair mismatch were used for Styl, BsrBI, MnlI and TaqI. The T1795A mutation destroys a HphI site. Amplified products were digested with HphI, HphI-resistant DNA bands excised from
10 the gel, sequenced and the sequence change identified.

SSCP analysis was performed as previously described (Williamson, M. P. et al Genes Chromosomes Cancer 92 (1994): 108-18).

15 Results

Initial RT-PCR Screen

RT-PCR of RNA from prostate cancer cell lines showed that plexins A1, A3 and B1 were expressed in prostate cells. The cDNA of the cytoplasmic domains of plexin A1, A3 and B1 was
20 sequenced in the prostate cancer cell lines PC3, DU145, LNCaP and benign prostate cell line Pre2.8.

A single nucleotide change (A to G) was observed in plexin B1 in LNCaP cDNA at nucleotide 5359, which potentially
25 changes threonine 1697 to an alanine. The sequence change is heterozygous in LNCaP cells. Karyotyping of LNCaP has revealed that this cell line has three copies of chromosome 3p. Estimation of the relative intensities of the mutant and wild type bands indicated that the mutation was present
30 in two of the three copies of chromosome 3p. PlexinB1 is

overexpressed in LNCaP as shown by quantitative RT-PCR (Figure 1).

The sequence change was absent from 120 control chromosomes
5 of similar genetic background to LNCaP.

DNA from 25 prostate tumours was extracted from paraffin sections and sequenced directly for the mutation found in LNCaP. The Thr1697Ala mutation was found in three of the
10 cancers, but was absent from seminal vesicle or lymph node tissue extracted from the same patient in the two cases where such tissue was available. This sequence change therefore represents a somatic change associated with prostate cancer.

15 Functional Analysis of PlexinB1 Mutation

The A5359G sequence change was introduced into a plexin B1 clone by in vitro mutagenesis. The mutant clone was expressed in NIH3T3 cells. Expression of the transfected clone and the presence of the introduced mutation was
20 confirmed by RTPCR and sequencing. No other sequence change was found in the cytoplasmic domain of the mutant clones.

NIH3T3 cells transfected with pcDNA3(PlexB1 (A5359G)) produced many colonies in soft agarose that were clearly
25 visible in 3 days and subsequently grew into many large colonies. Significantly fewer colonies were seen for NIH3T3 cells transfected with plexinB1 of wild type sequence, and no colonies were seen for NIH3T3 cells transfected with the parent or vector control after one month (Figure 2). Growth
30 in soft agarose was observed in all three mutant clones from two independent transfections that were tested.

Therefore the Thr1697Ala sequence change confers anchorage independent growth to NIH3T3 cells.

Additional Mutations

80 primary prostate tumours (including 10 of the 25
5 providing DNA for sequencing the mutation seen in LNCaP)
and 11 prostate cancer metastases were screened for
mutations in the cytoplasmic domain of the plexinB1 gene by
SSCP analysis. Aberrant bands were excised from the gel and
sequenced directly. 12 additional mis-sense mutations were
10 identified (Table 1, Figure 3).

One of these mutations, A5653G, which potentially changes
the threonine at position 1795 to an alanine, was found in
7/11 (64%) of metastases and 33/80 (41%) of primary
15 cancers. The presence of the T1795A mutation was confirmed
by direct sequencing of DNA from the metastases (Figure 4)
and by restriction enzyme digestion in the 33 primary
tumours.

20 Position 1795 is part of a potential serine/threonine
kinase phosphorylation site which is highly conserved in
evolution. A threonine or serine is present at this
position in the protein in mouse, Drosophila and C.elegans
plexinB1 homologues as well as members of the other classes
25 of the plexin family of proteins (plexins A1, A2, A3, B2,
B3 and D1) (Figure 5).

A T5714C sequence change, predicted to result in a change
of amino acid from leucine 1815 to proline was found in 3
30 metastases. A leucine or valine in this position is
conserved in plexinB1 homologues in other species and in
other plexin family members (Figure 5).

A C5060T mutation, which potentially changes proline 1597 to leucine, was also found in 3 metastases and a second mutation in a primary tumour (C5059T) changes the same amino acid to serine. A proline at this position in the protein is not conserved during evolution (Figure 5)).

Six further mutations were identified in single metastases: G5074A (Gly1602T), T5468C (Thr1733Ile), A5474G (Asn1735Ser), C5662T (Pro1798Ser), A5674G (Thr1802Ala) and C5980T (Arg1904Trp). Thr1733 and Pro1798 are conserved in both A and B class plexins, while Gly1602, Asn1735, Arg1904 are conserved in class B plexins. Thr1802 is replaced with acidic amino acids in A class plexins which may mimic a phosphorylation site. Finally two mutations (F1711I and T1776A) were found in single primary tumours. Phe1711 is conserved in class B plexins and Thr1776 is conserved in class A and B plexins.

All 12 additional mutations were found to be absent from normal tissue of the same patient where available and are therefore somatic. The sequence changes were absent from at least 50 control individuals in every case.

The present findings show that mutation of the plexinB1 gene is associated with prostate cancer progression. The incidence of mutations was higher in prostate cancer metastases than primary tumours, with mutations in 91% of metastases (10/11) and 45% (36/80) of primary tumours. The metastases frequently contained multiple mutations. Three of the metastases each contained 3 mutations within this short stretch of the plexinB1 gene and 3 others contained two mutations. 8 of the mutations (L1815P in 3 tumours and

P1798L in 3 tumours, T1802A and Pro1798Ser in one tumour each) occurred in addition to the common Thr1795Ala change. T1802A was found in the same SSCP band as the common (T1795A) mutation indicating that the two mutations are on the same allele. In contrast, only single mutations were found in primary tumours. In addition, the proportion of cells containing mutant plexinB1 was higher in metastases than primary tumour tissue. In one case where both primary and metastatic tissues were available from the same patient, the mutant allele was more highly represented in the metastases than the primary tumour. Together these results provide indication that mutations in the plexinB1 gene are selected for during cancer metastasis.

Tumourgenicity of mutant PlexinB1

The three mutations most frequently found in prostate cancer were A5359G, A5653G, and T5714C. NIH3T3 cells expressing wild type or mutant plexinB1 (A5359G, A5653G, or T5714C) were tested for tumourigenicity in athymic nude mice. A significant reduction in tumour growth was seen between parental NIH3T3 cells or cells transfected with empty vector and wild type plexinB1 ($p \leq 0.01$; 2-tailed Mann-Whitney U test). Parental NIH3T3 cells or cells transfected with empty vector formed tumours in 8/10 sites within 20 days. In contrast, cells transfected with wild type plexinB1 produced tumours at a greatly reduced rate in one out of 10 sites after 40 days and 3 out of 10 sites by 90 days. The 3 mutant constructs produced tumours at a significantly faster rate than WT plexinB1 ($p \leq 0.05$; 2-tailed Mann-Whitney U test, Figures 6 and 7). The observation that expression of wild type plexinB1 suppresses tumour formation provides indication that plexinB1 acts as a tumour suppressor gene. The mutations A5359G, A5653G, and

T5714C partially counteracted the reduction in tumour take rate, indicating that these mutations abrogate the tumour suppressor function of plexinB1.

- 5 Expression of wild type and mutant plexin B1 constructs tagged with GFP in NIH3T3 cells showed that the mutations do not to influence the sub-cellular distribution of the protein.

10 PlexinB1 Mutations in Breast Cancer

30 breast cancer lymph node metastases were screened for mutations in exons 22-29 of the plexin B1 gene by SSCP and 7 mutations were found in 6 cases (Table 2, Figure 8).

- Three metastases had a T5059C sequence change, which is
15 predicted to alter Pro1597 to Ser, and one metastasis had a mutation in the adjacent nucleotide (C5060T), which alters the same amino acid to Leu. Both these mutations were also observed in prostate cancer metastases. One breast cancer metastasis had the sequence change C1713T, which changes
20 Leu1815 to Phe. Leu1815 was altered to Pro in 3 cases of prostate cancer metastasis. Two of the mutations were only found in breast cancer metastasis: G5452A (Gly1728Ser) and G5458A (Ala1730Thr). G5452A and C1713T were found in the same metastasis. These mutations were not present in 100
25 control chromosomes. These mutations were present at a low copy number in the lymph node metastasis.

Activity of Mutant PlexinB1

- The mutation A5359G, which confers a T1697A mutation in the plexinB1 polypeptide, was found to confer anchorage
30 independent growth on NIH3T3 cells. This mutation therefore confers a gain of function. LNCaP cells, which contain this

mutation, were observed to produce enhanced levels of the mutant mRNA and increased levels of unprocessed protein.

Although the precise effect of the identified mutations on downstream signalling is unknown, the Thr1697Ala change was found to confer anchorage independent growth in vitro. Five of the other identified mutations abolish a potential serine/threonine kinase phosphorylation site.

Phosphorylation of these specific serine/threonine kinase sites may have an inhibitory effect on plexinB1 activity. Three of the mutations result in loss of a proline residue, which is likely to affect the structure of the receptor.

Leu1815 is also highly conserved and forms part of Leu Ile Leu motif which is known to regulate transmembrane receptor internalisation and down-regulation.

Nucleotide	Amino acid	somatic +'	primary(95)	metastases(11)										Total
				1	2	3	4	5	6	7	8	9	10	
C5060T	P1597L	X			X	X		X	X					4
C5059T	P1597S	ND	1											1
G5074A	G1602T	X							X					1
A5359G*	T1697A*	X	3											3
T5401A	F1711I	ND	1											1
C5468T	T1733I	X								X				1
A5474G	N1735S	X								X				1
A5596G	T1776A	ND	1											1
A5653G	T1795A	X	33	X	X	X	X	X			X	X		40
C5662T	P1798S	ND										X		1
A5674G	T1802A	X				X								1
T5714C	L1815P	X			X			X			X			3
C5980T	R1904W	ND											X	1
			(39/95=41%)	(10/11=91%)										59

* T1697A mutation was found in LNCaP and 3 primary tumours by direct sequencing

+' mutation absent from non cancer tissue from the same patient

Table 1

		metastases(30)						Total
		1	2	3	4	5	6	
T5059C	P1597S	x	x	x				3
C5060T	P1597L				x			1
C5713T	L1815F					x		1
G5452A	G1728S					x		1
G5458A	A1730T						x	1
								7

Table 2

Claims:

1. A method of assessing an individual for a cancer condition comprising;

5 providing a tissue sample obtained from said individual,

determining the presence in said sample of one or more cells comprising a plexinB1 nucleic acid sequence having one or more mutations therein,

10 the presence of said one or more cells being indicative of said individual having a cancer condition.

2. A method according to claim 1 wherein said one or more mutations alter the expression and/or activity of a plexin
15 polypeptide encoded by said nucleic acid.

3. A method according to any one of the preceding claims wherein said one or more mutations are in a non-coding region of said plexin nucleic acid.

20

4. A method according to claim 1 or claim 2 wherein said one or more mutations are in a coding region of said plexin nucleic acid.

25 5. A method according to claim 4 wherein said plexin nucleic acid sequence encodes a plexinB1 polypeptide having one or more mutations therein.

6. A method according to claim 5 wherein the plexin B1
30 polypeptide comprises one or more mutations in the cytoplasmic domain thereof.

7. A method according to claim 5 or claim 6 wherein the one or more mutations are at one or more mutation sites selected from the group consisting of T1697, T1733, T1776, T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735,
5 R1904, A1730, G1728, and K1613.

8. A method according to claim 7 wherein the one or more mutations are selected from the group consisting of T1697A, T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S,
10 F1711I, G1602T, L1815P, N1735S, R1904W, A1730T, G1728S, L1815F, and K1613E.

9. A method according to claim 8 wherein the polypeptide comprises a mutation selected from the group consisting of
15 T1795A, P1597S, P1597L, and L1815P.

10. A method according to claim 8 wherein the polypeptide comprises the mutation T1795A.

20 11. A method according to any one of the preceding claims wherein the cancer condition is prostate cancer or breast cancer.

12. A method according to any one of claims 1 to 11
25 wherein the presence of said one or more cells is determined by detecting the presence of said plexinB1 polypeptide.

13. A method of determining the invasiveness of a cancer
30 cell in a sample obtained from an individual, the method comprising,

determining the presence or absence in said cell of a plexin B1 polypeptide having one or more mutations therein,

the presence of said plexin B1 polypeptide being indicative that the cell is invasive.

14. A method according to claim 13 wherein said one or
5 more mutations alter the expression and/or activity of a plexin polypeptide encoded by said nucleic acid.

15. A method according to claim 13 or claim 14 wherein
said one or more mutations are in a non-coding region of
10 said plexin nucleic acid.

16. A method according to claim 13 or claim 14 wherein
said one or more mutations are in a coding region of said
plexin nucleic acid.

15

17. A method according to claim 16 wherein said plexinB1
nucleic acid sequence encodes a plexinB1 polypeptide having
one or more mutations therein.

20 18. A method according to claim 17 wherein the plexinB1
polypeptide comprises one or more mutations in the
cytoplasmic domain thereof.

19. A method according to claim 17 or claim 18 wherein the
25 one or more mutations are at one or more mutation sites
selected from the group consisting of T1697, T1733, T1776,
T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735,
R1904, A1730, G1728, and K1613.

30 20. A method according to claim 19 wherein the one or more
mutations are selected from the group consisting of T1697A,
T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S,

F1711I, G1602T, L1815P, N1735S, R1904W, A1730T, G1728S, L1815F, and K1613E.

21. A method according to claim 20 wherein the polypeptide
5 comprises a mutation selected from the group consisting of T1795A, P1597L, P1597S, and L1815P.

22. A method according to claim 19 wherein the polypeptide
comprises the mutation T1795A.
10

23. A method according to any one of the preceding claims
wherein the cancer condition is prostate cancer or breast
cancer.

15 24. A method according to any one of claims 13 to 23
wherein the presence of said one or more cells is
determined by detecting the presence of said plexinB1
polypeptide.

20 25. A method of identifying and/or obtaining a putative
anti-cancer agent, the method comprising;
contacting a plexinB1 polypeptide with a test compound
and;
determining the activity of the plexinB1 polypeptide
25 in the presence relative to the absence of test compound.

26. A method according to claim 25 wherein plexin
polypeptide comprises one or more mutations.

30 27. A method according to claim 26 wherein the one or more
mutations are in the cytoplasmic domain of the plexinB1
polypeptide.

28. A method according to claim 27 wherein the one or more mutations are at one or more mutation sites selected from the group consisting of T1697, T1733, T1776, T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735, R1904, A1730,
5 G1728, and K1613.

29. A method according to claim 28 wherein the one or more mutations are selected from the group consisting of T1697A, T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S,
10 F1711I, G1602T, L1815P, N1735S, R1904W, A1730T, G1728S, L1815F, and K1613E.

30. A method according to any one of claims 25 to 29 wherein the plexinB1 polypeptide is expressed on the
15 surface of a cell

31. A method according to claim 30 wherein the activity is plexinB1-mediated anchorage independent growth of said cell.
20

32. A method according to any one of claims 25 to 30 wherein the activity of the plexinB1 polypeptide is determined by determining the binding of said polypeptide to one or more of semaphorin4D, active Rac1, neuropilin,
25 PDZ-RhoGEF and LARG and other components of the semaphorin signalling pathway interacting with plexinB1.

33. A method according to any one of claims 25 to 30 wherein the activity of the plexinB1 polypeptide is
30 determined by determining the activation of Rho GTPase.

34. A method according to any one of claims 26 to 30 comprising the further step of;

contacting a wild-type plexinB1 polypeptide with the test compound, and;

determining the activity of the wild-type plexinB1 polypeptide.

5

35. A method according to any one of claims 26 to 30 comprising the further steps of;

contacting the mutant plexinB1 polypeptide with the test compound in the presence of a wild-type plexinB1, and;

10 determining the activity of the wild-type plexinB1 polypeptide.

36. A method of identifying and/or obtaining a compound as a putative anti-cancer agent, the method comprising;

15 contacting a plexinB1 nucleic acid with a test compound and;

determining the expression of the plexinB1 nucleic acid in the presence relative to the absence of test compound.

20 37. A method according to claim 36 wherein the plexinB1 nucleic acid comprises one or more mutations.

38. A method according to claim 37 wherein one or more mutations are in a non-coding region of the nucleic acid.

25

39. A method according to claim 37 wherein the one or more mutations are in a coding region of the nucleic acid.

30 40. A method according to claim 39 wherein the one or more mutations are in a region of the nucleic acid which encodes the cytoplasmic domain of the plexinB1 polypeptide.

41. A method according to claim 40 wherein the one or more mutations are at one or more mutation sites selected from the group consisting of 5059, 5060, 5074, 5107, 5359, 5401, 5452, 5458, 5468, 5474, 5596, 5653, 5662, 5674, 5713, 5714, 5 and 5980 of the plexinB1 coding sequence.

42. A method according to claim 42 wherein the one or more mutations are selected from the group consisting of C5059T, C5060T, G5074A, A5107G, A5359G, T5401A, G5452A, G5458A, 10 T5468C, A5474G, A5596G, A5653G, C5662T, A5674G, C5713T, T5714C and C5980T.

43. A method according to claim 36 comprising determining the increase in the expression of wild-type plexin B1 in 15 the presence of said test compound.

44. A method according to any one of claims 36 to 41 comprising determining the decrease in the expression of mutant plexin B1 in the presence of said test compound

20

45. A method according to any one of claims 26 to 44 comprising identifying the test compound as a putative anti-cancer agent.

25 46. A method according to claim 45 comprising isolating and/or purifying the test compound.

47. A method according to claim 46 comprising modifying the test compound to optimise its pharmaceutical 30 properties.

48. A method according to any one of claims 45 to 47 comprising formulating said test compound with a pharmaceutically acceptable excipient.

- 5 49. A method of producing a pharmaceutical compound comprising the steps of;
identifying a compound using any one of claims 25-47 and;
formulating said test compound with a pharmaceutically acceptable excipient.
- 10 50. A compound identified as a putative anti-cancer agent by a method of claim 45.
- 15 51. A pharmaceutical composition comprising the compound of claim 50 and a pharmaceutically acceptable excipient.
52. A compound according to claim 50 for use in a method of treatment
- 20 53. Use of a compound according to claim 50 in the manufacture of a medicament for use in the treatment of cancer.
- 25 54. A nucleic acid encoding plexinB1 or its complement or a fragment thereof for use in a method of treatment.
55. A nucleic acid encoding mutant plexinB1 polypeptide or its complement or a fragment thereof for use in a method of treatment.
- 30 56. A nucleic acid according to claim 55 wherein the mutant plexinB1 polypeptide has one or more mutations in the cytoplasmic domain.

57. A nucleic acid according to claim 56 wherein the one or more mutations are at one or more mutation sites selected from the group consisting of T1697, T1733, T1776,
5 T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735, R1904, A1730, G1728, and K1613.

58. A nucleic acid according to claim 57 wherein the one or more mutations are selected from the group consisting of
10 T1697A, T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S, F1711I, G1602T, L1815P, N1735S R1904W, A1730T, G1728S, L1815F, and K1613E

59. A nucleic acid according to any one of claims 54 to 58
15 for use in a method of treating cancer.

60. Use of nucleic acid encoding plexinB1 or its complement or a fragment thereof in the manufacture of a medicament for the treatment of cancer.

20 61. Use of nucleic acid encoding mutant plexinB1 or its complement or a fragment thereof in the manufacture of a medicament for the treatment of cancer.

25 62. Use according to claim 61 wherein the mutant plexinB1 polypeptide has one or more mutations in the cytoplasmic domain.

30 63. Use according to claim 61 wherein the one or more mutations are at one or more mutation sites selected from the group consisting of T1697, T1733, T1776, T1795, T1802, P1597, P1798, F1711, G1602, L1815, N1735, R1904, A1730, G1728, and K1613.

64. Use according to claim 63 wherein the one or more mutations are selected from the group consisting of T1697A, T1733I, T1776A, T1795A, T1802A, P1597L, P1597S, P1798S,
5 F1711I, G1602T, L1815P, N1735S, R1904W, A1730T, G1728S, L1815F and K1613E.

65. A method of treating a cancer condition in an individual, the method comprising introducing nucleic acid
10 according to claims 60-64 in to one or more cells of said individual.

66. A method of screening for an antibody molecule specific for a mutant plexinB1, the method comprising;
15 providing a population of antibody molecules specific for mutant plexinB1,
contacting said population with a normal plexinB1 polypeptide,
identifying one or more members of said population which
20 bind preferentially to mutant plexinB1 relative to normal plexinB1.

67. A antibody molecule which specifically binds to a mutant plexinB1 polypeptide.
25

68. A method of treating a cancer condition in an individual, the method comprising reducing the activity of mutant plexinB1 polypeptide in one or more cells of said individual.
30

69. A method according to claim 68 wherein the activity of mutant plexinB1 polypeptide is reduced by administering an antagonist of mutant plexinB1 to said individual.

70. A method according to claim 68 wherein the activity of mutant plexinB1 polypeptide is reduced by decreasing or abolishing expression of the mutant plexin B1 polypeptide.

5

71. A method according to claim 68 wherein expression of the mutant plexin B1 polypeptide is abolished or reducing by administering a nucleic acid according to any one of claims 53 to 58.

10

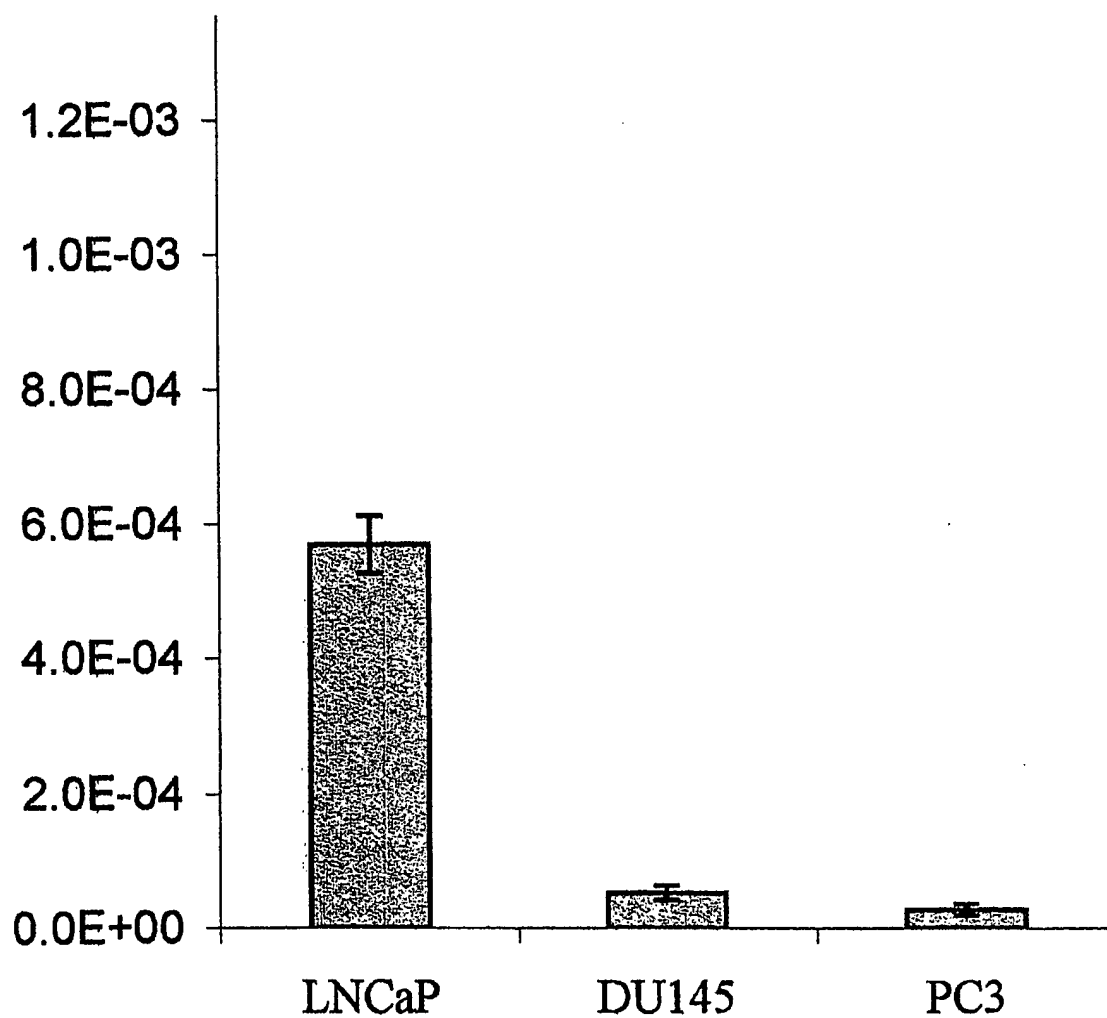
72. A method of reducing the invasiveness of a tumour in an individual comprising reducing the activity of mutant plexinB1 polypeptide in one or more cells of said tumour.

15 73. A method according to claim 72 wherein the activity of mutant plexinB1 polypeptide is reduced by administering an antagonist of mutant plexinB1 to said individual.

20 74. A method according to claim 72 wherein the activity of mutant plexinB1 polypeptide is reduced by decreasing or abolishing expression of a mutant plexinB1 polypeptide.

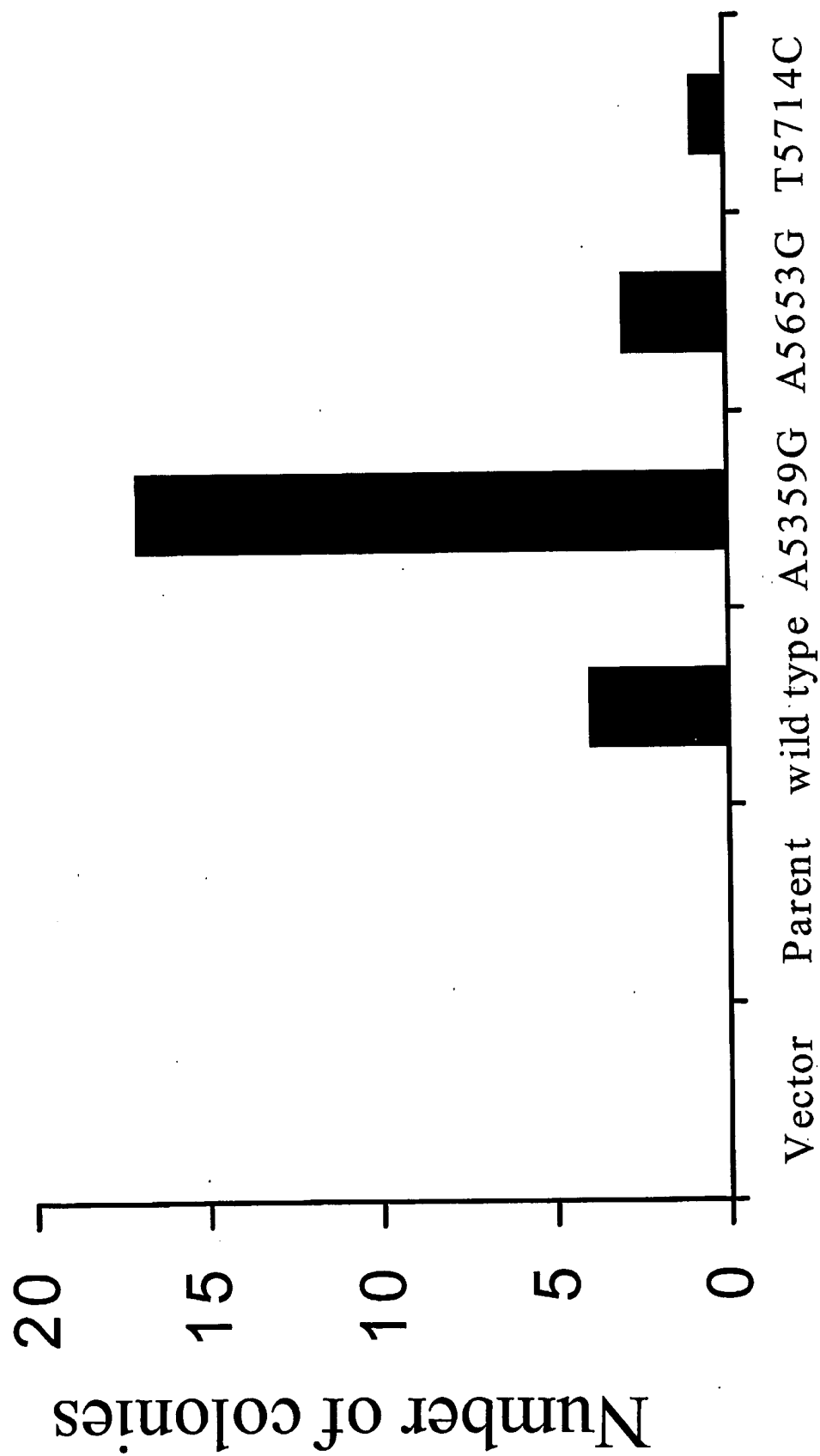
25 75. A method according to claim 72 wherein expression of a mutant plexin B1 polypeptide is abolished or reducing by administering a nucleic acid according to any one of claims 53 to 58.

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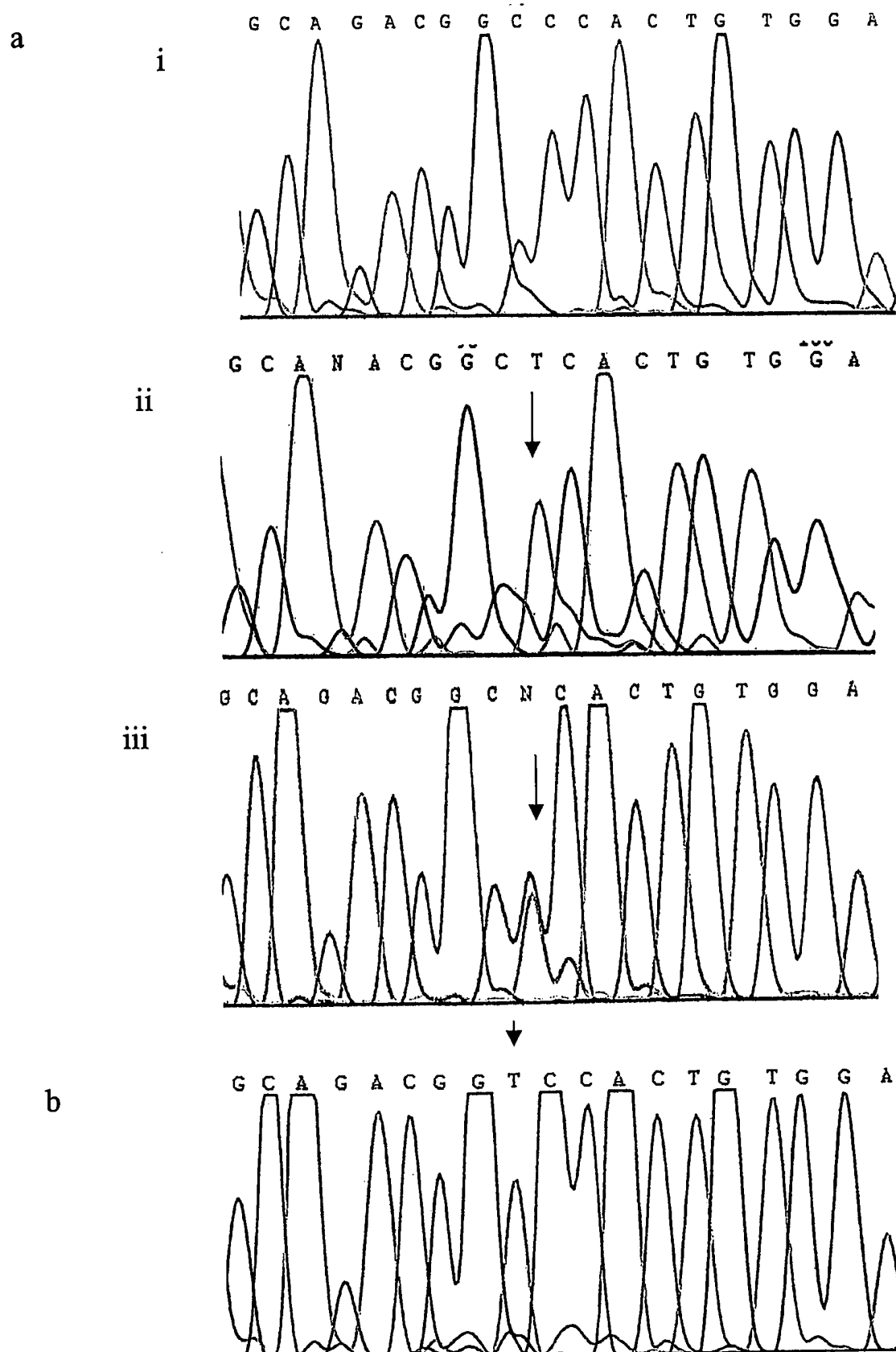
Figure 1

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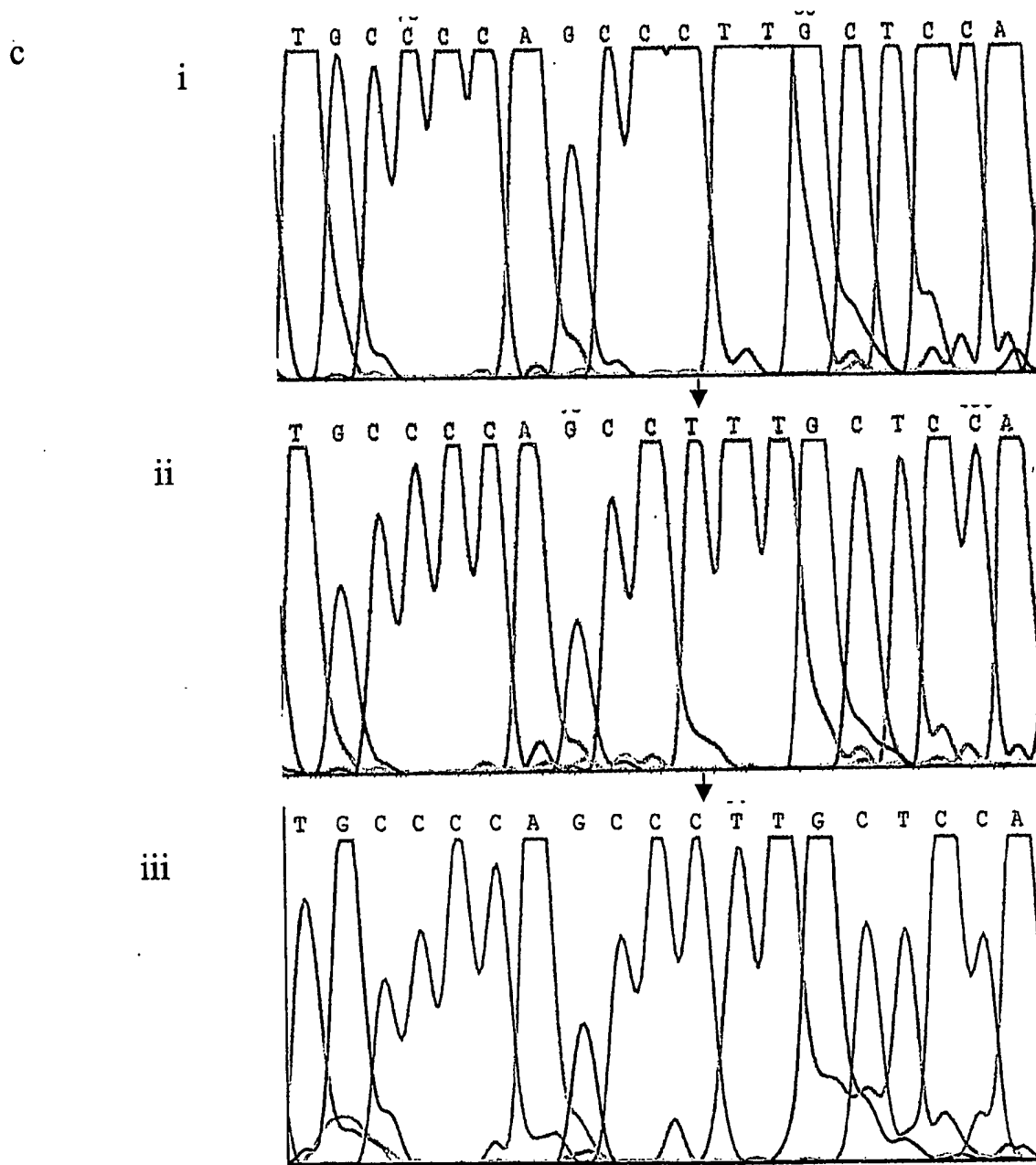
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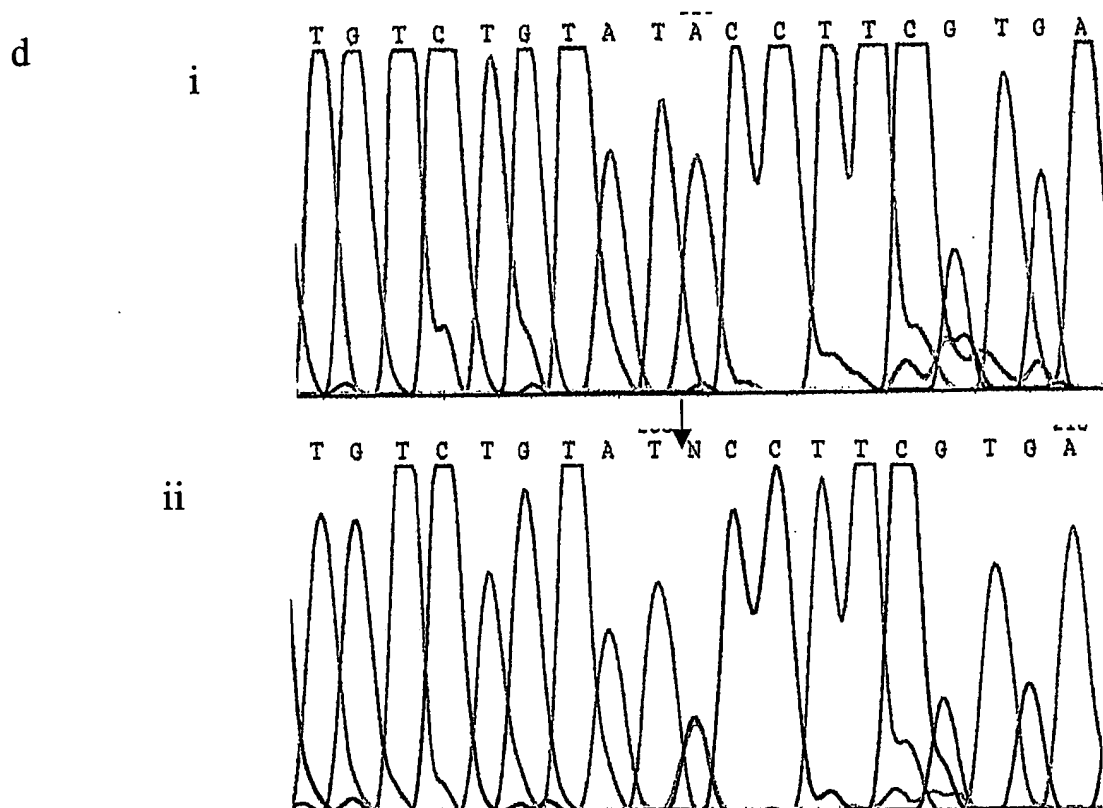
3/19

Figure 3

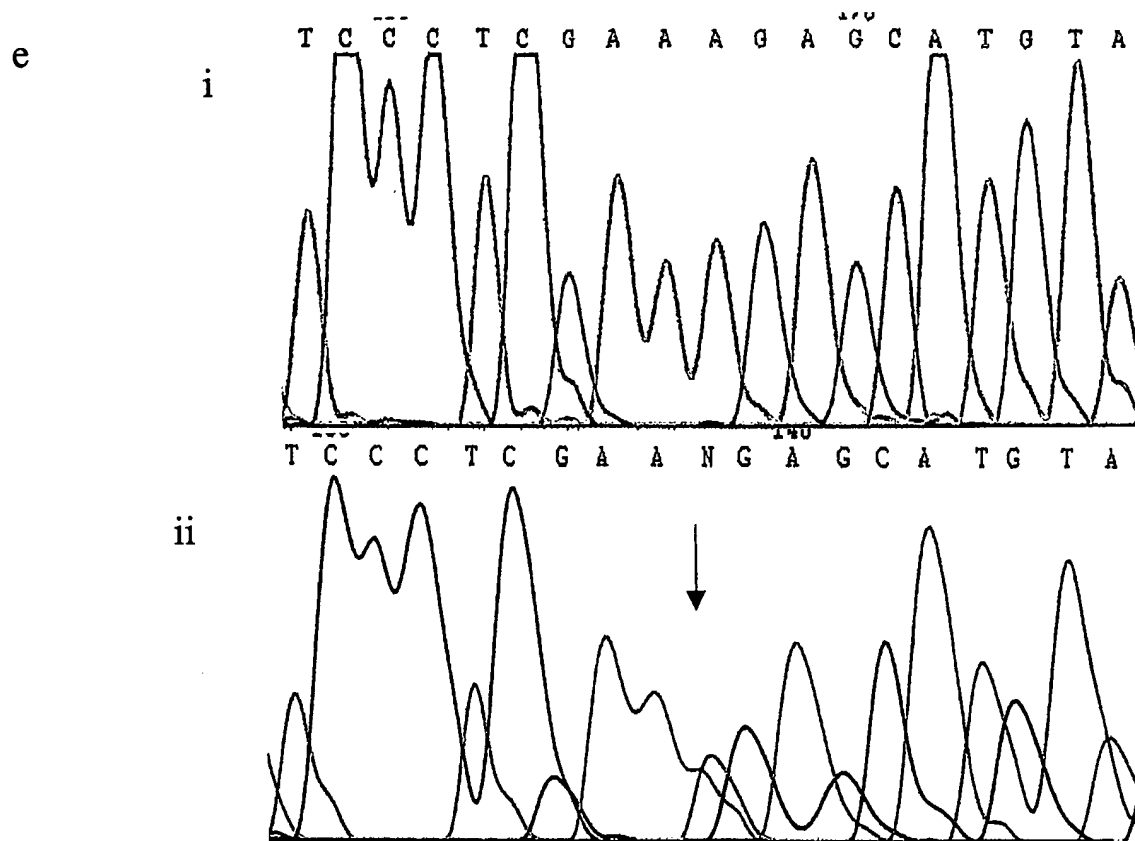
4/19

Figure 3 (Continued)

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Figure 3 (Continued)

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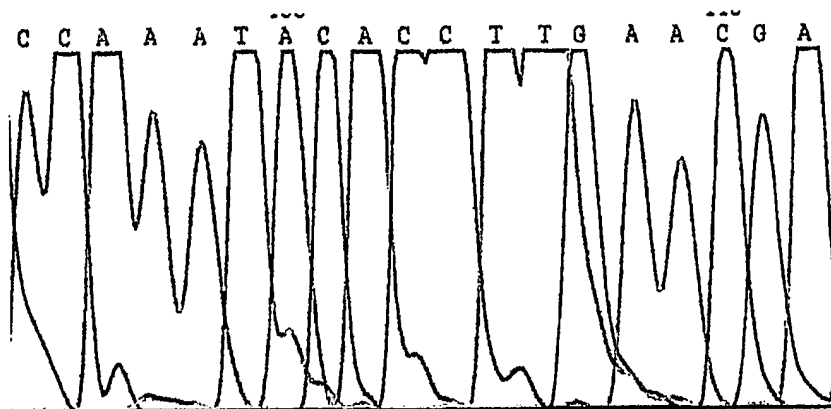
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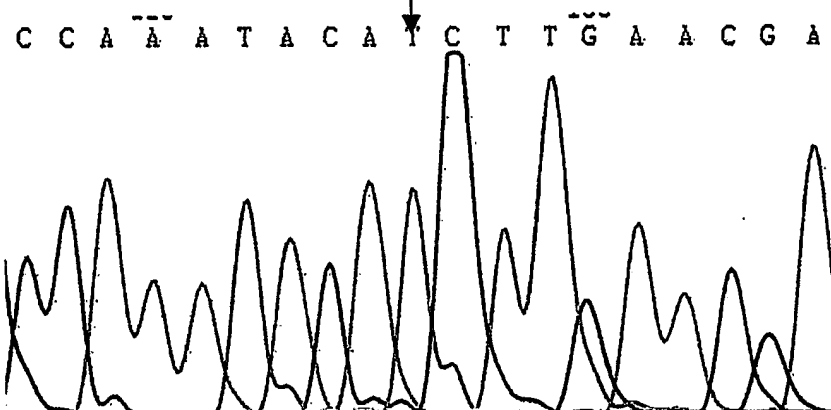
Figure 3 (Continued)

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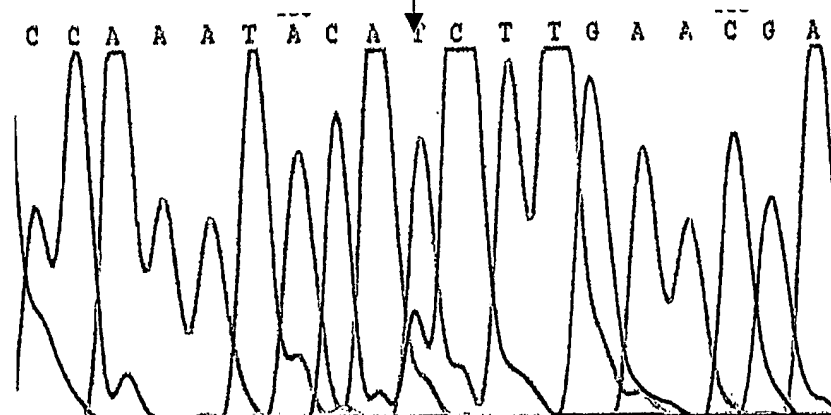
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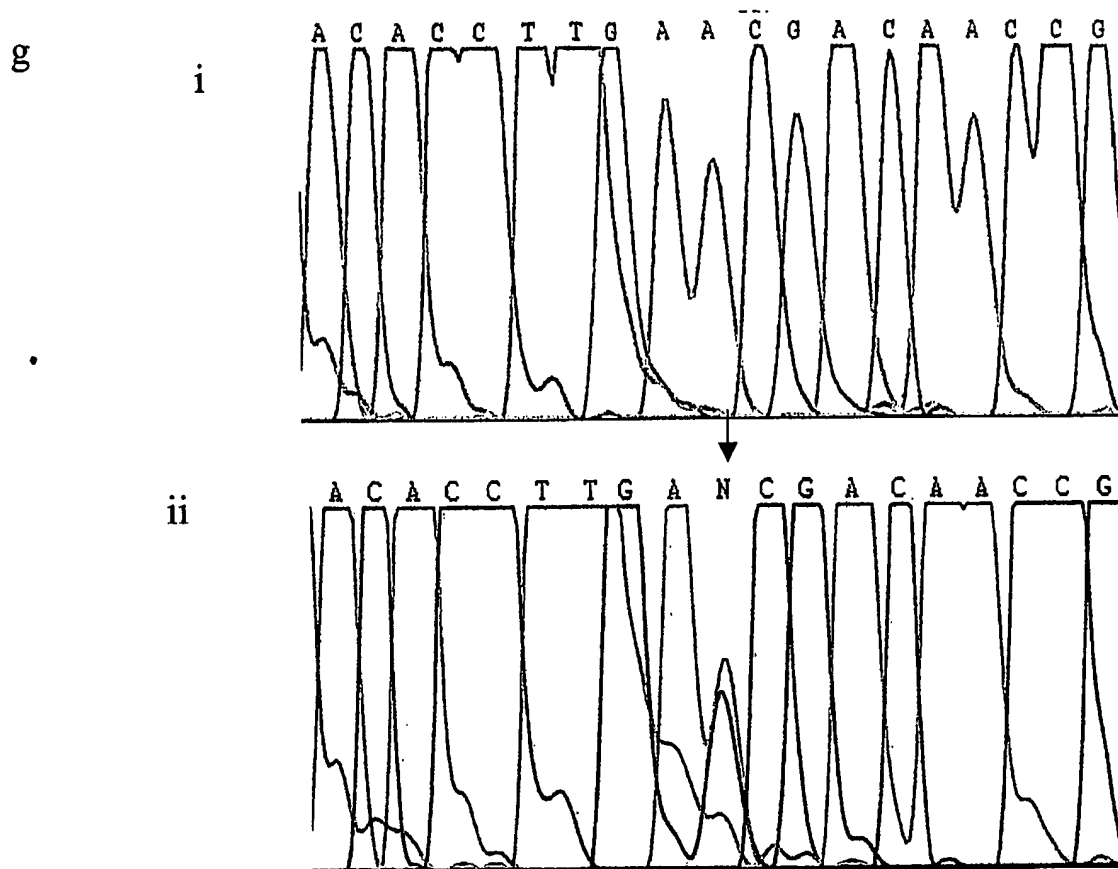
ii



iii



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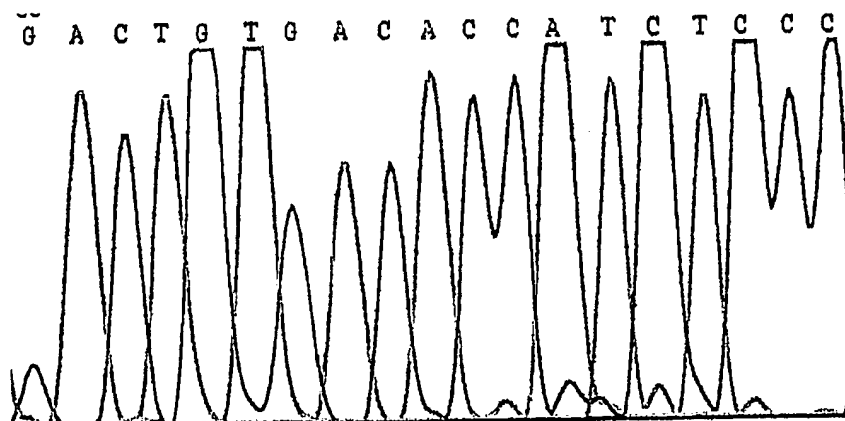
Figure 3 (Continued)

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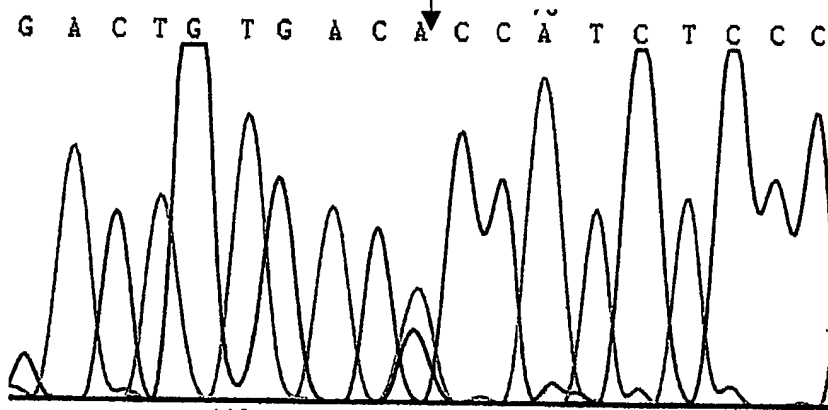
Figure 3 (Continued)

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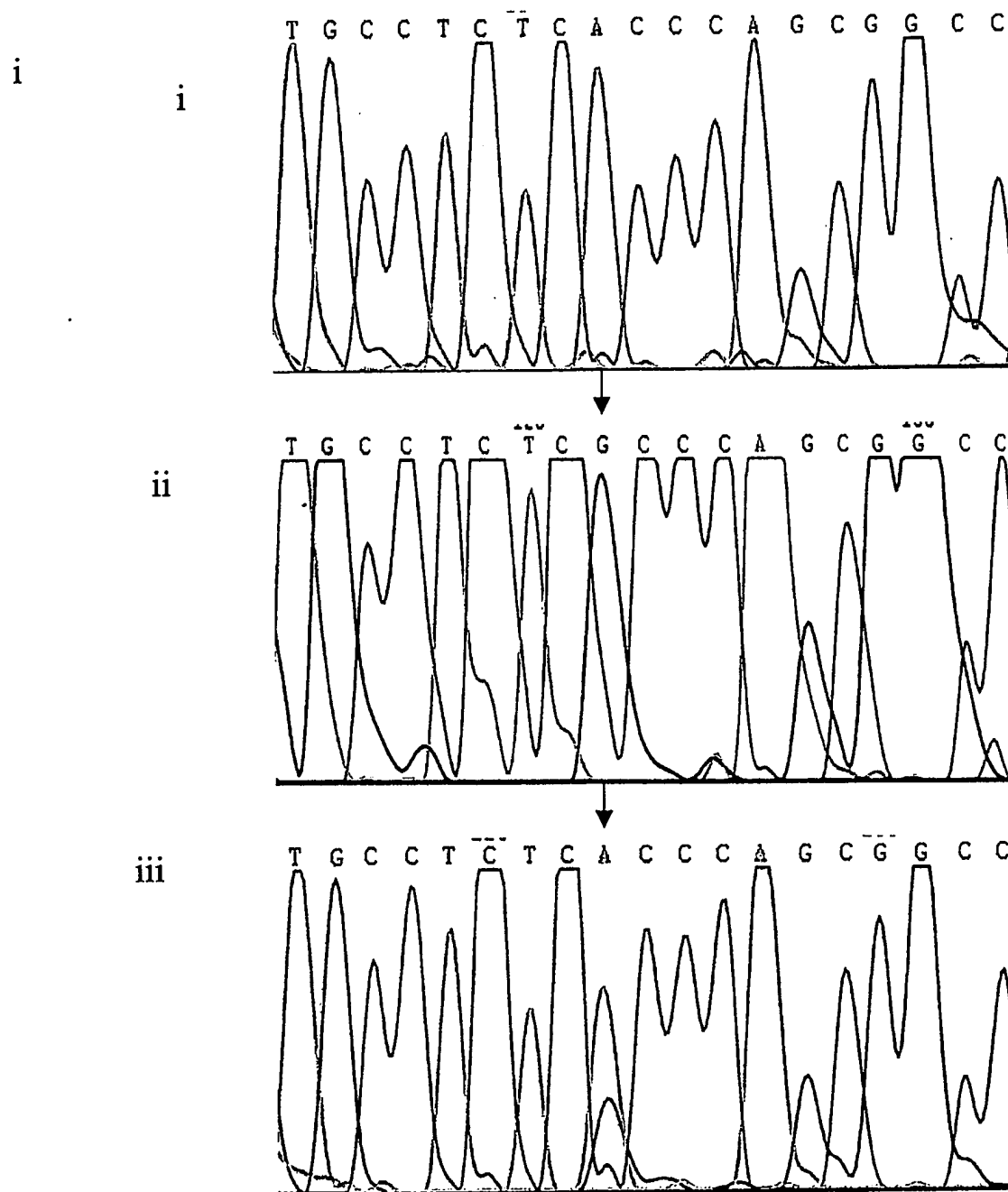
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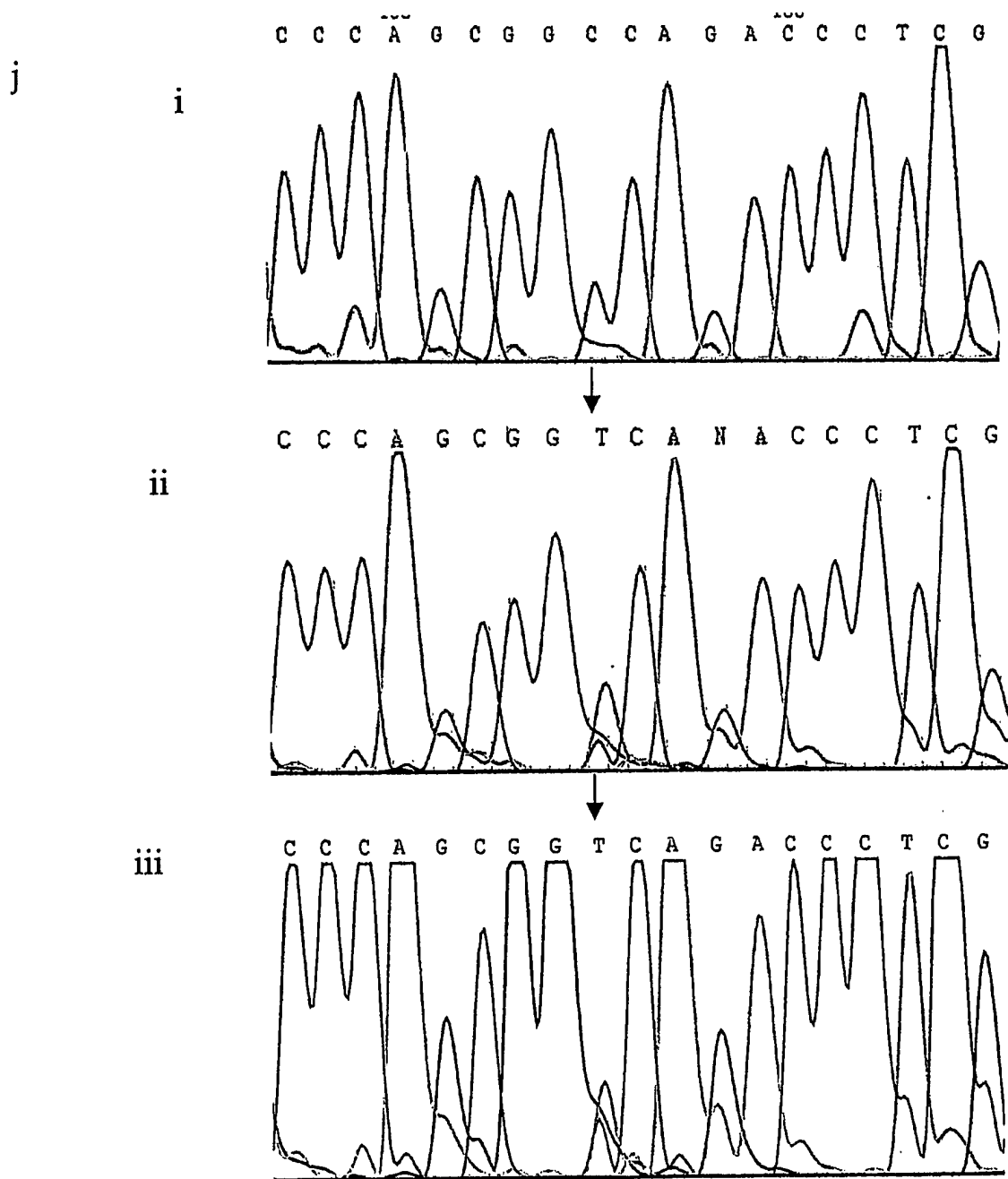
ii



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Figure 3 (Continued)

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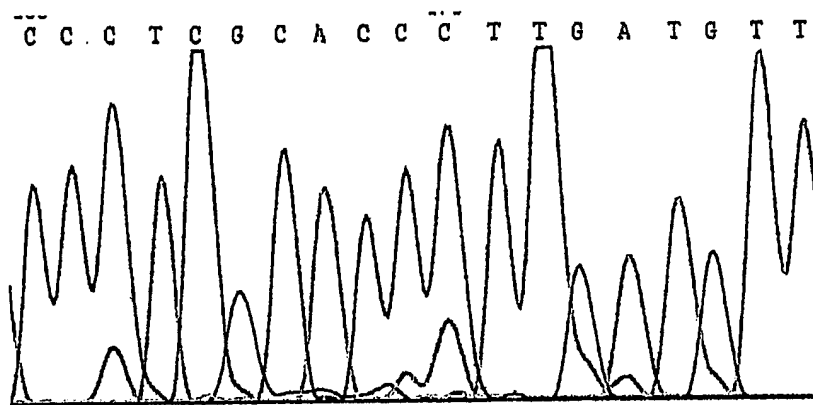
Figure 3 (Continued)

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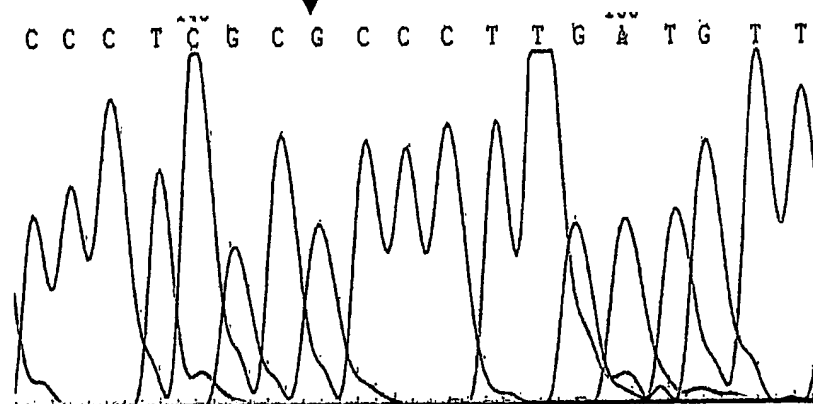
Figure 3 (Continued)

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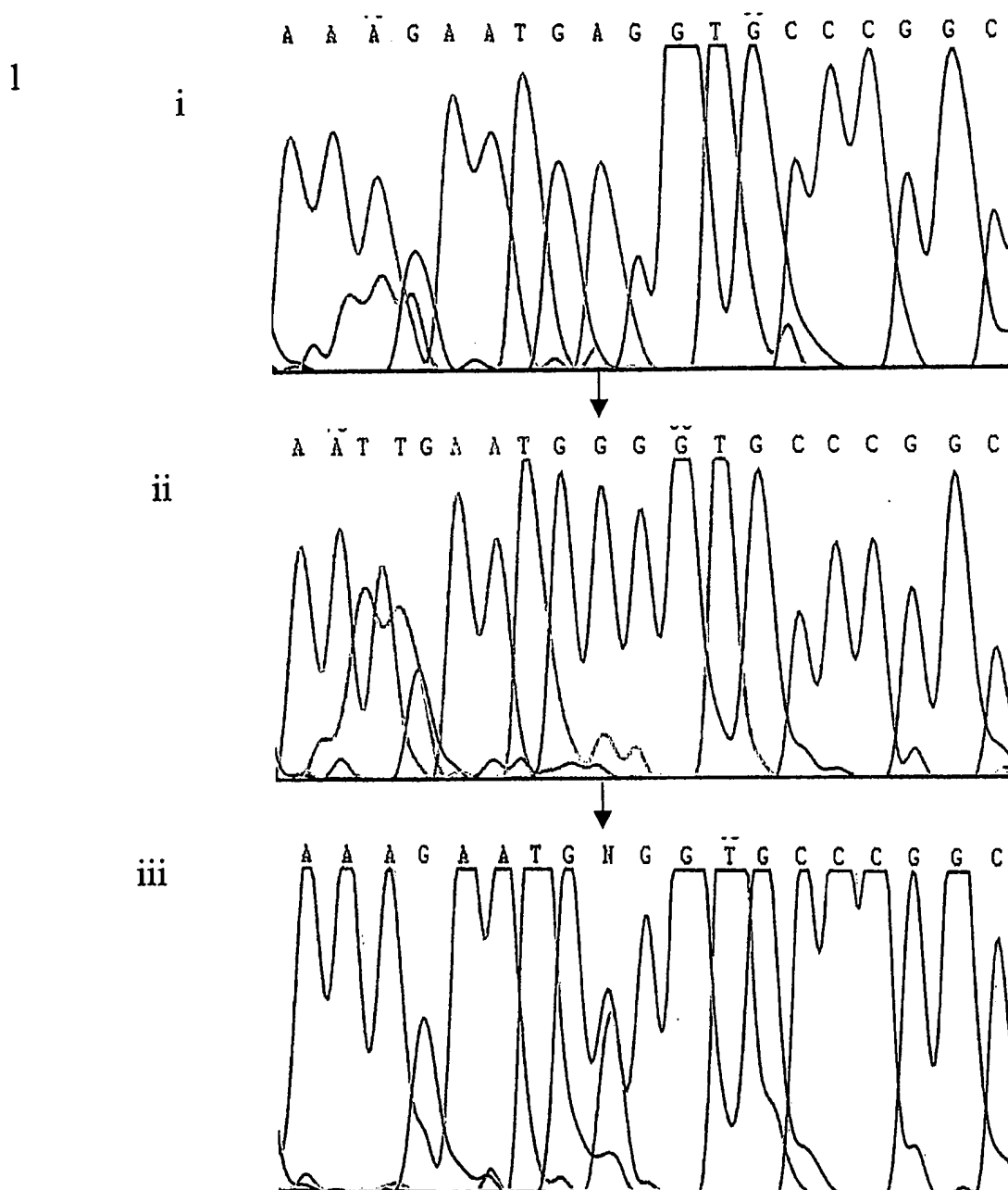
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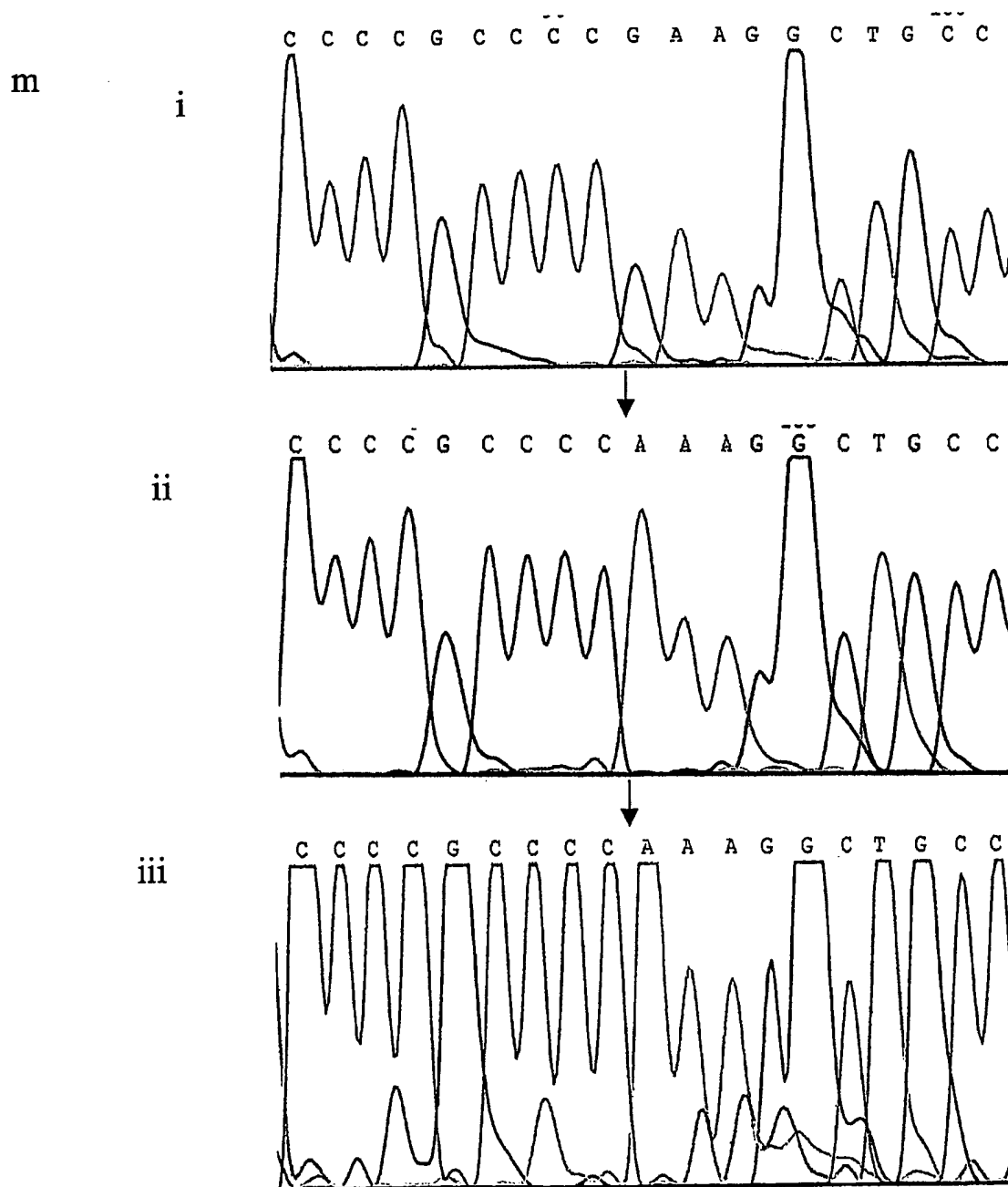
ii



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Figure 3 (Continued)

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Figure 3 (Continued)

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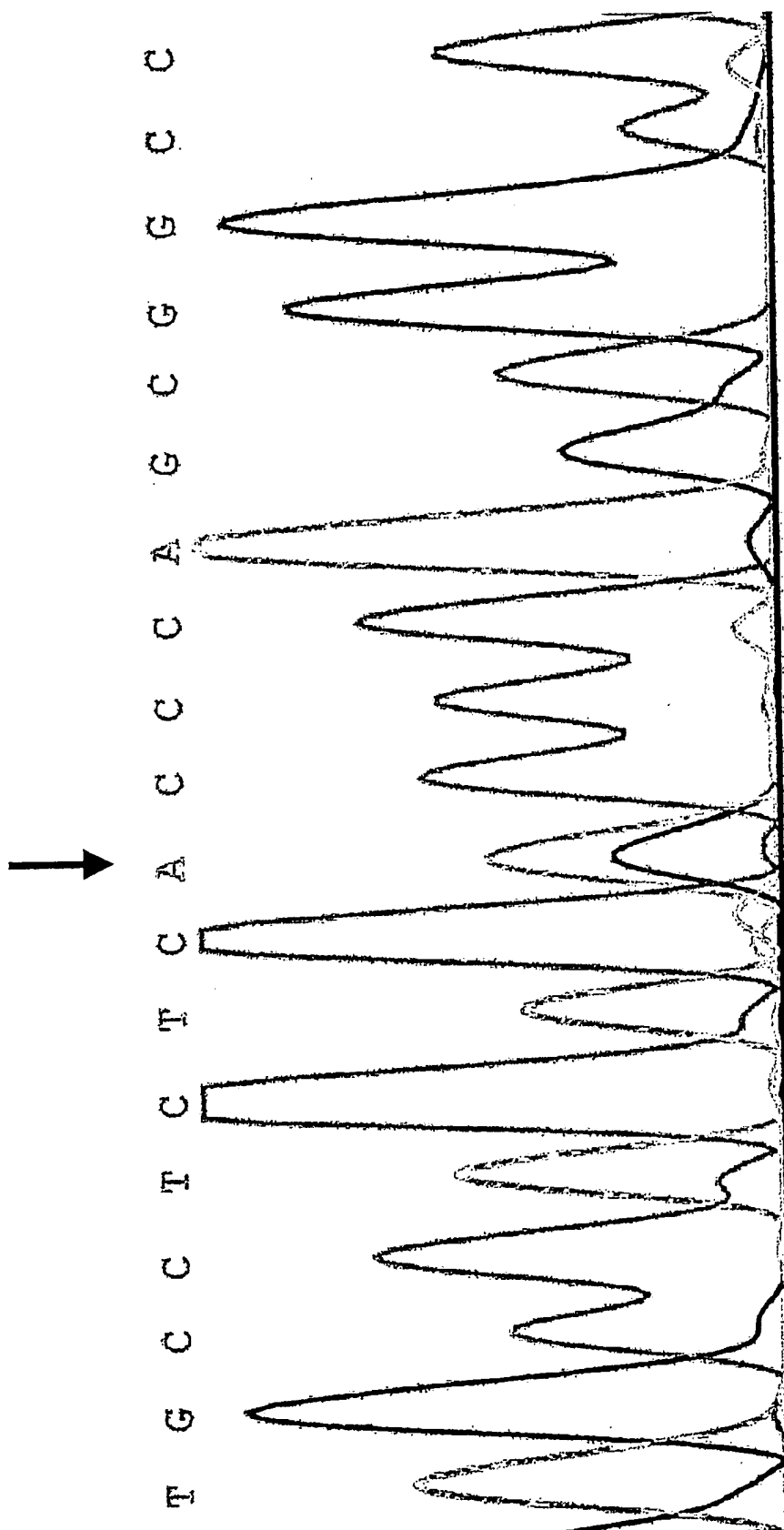


Figure 4

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	I I I L I I	
	G G A A A A	
	R R K K R K	
1711	F F F Y F F	
	L L L L L L	
	M M L M K	
	Y Y F Y Y Y	
	L L L F L L	
	D D E P E D E	
	R R K T R K K	
	V V L L L L L	
	F F Y H F Y F	
1697	T T D S A Q K A	
	Y Y Y Y Y H	
	L L M L L L L	
	C C C C C L	
	I I I I I F	
	Q Q Q Q Q L	
	G G V A T K L	
	L L M L L L	
1602	G G A T G A T	
	Q Q A V Q K A	
	E E D P R E K	
	V V Y L V V H	
	T T N T T T	
1597	P P T R A Q Q L	
	R R Q A C Q Q	
	R R P L H G G	
	S S S S G N	
	E D G P D G	
	Hs PLXNB1	
	Mm plxnbt	
	Dm plxnB	
	Ce plx-2	
	Hs PLXNB3	
	Hs PLXNB2	
	Hs PLXNA2	

	G G G G G G G G	
	S S S S C T S T T	
	- - - - - I - - - - - Y	
1776	T T T A T T T T T A	
	D D D D D D D D	
	C T W C T C C C	
	D D D A D N N D	
	L L N H L L L L	
	R R R K R G K K	
	N N E A S T D D	
	D D E E D D E E	
1735	N N S N N S S S	
	L L L I L L L	
1733	T T S T T T S S I	
	Y Y Y Y R Y Y Y	
	K K R R K K R R	
1730	A A A A A A A T	
	K K D D K K E E	
1728	G G N G G K G G S	
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	V V I V V V I I	
	S S A A V A A A	
	D D D D D D D	
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	Mm plxnB1	
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	Hs PLXNA1	

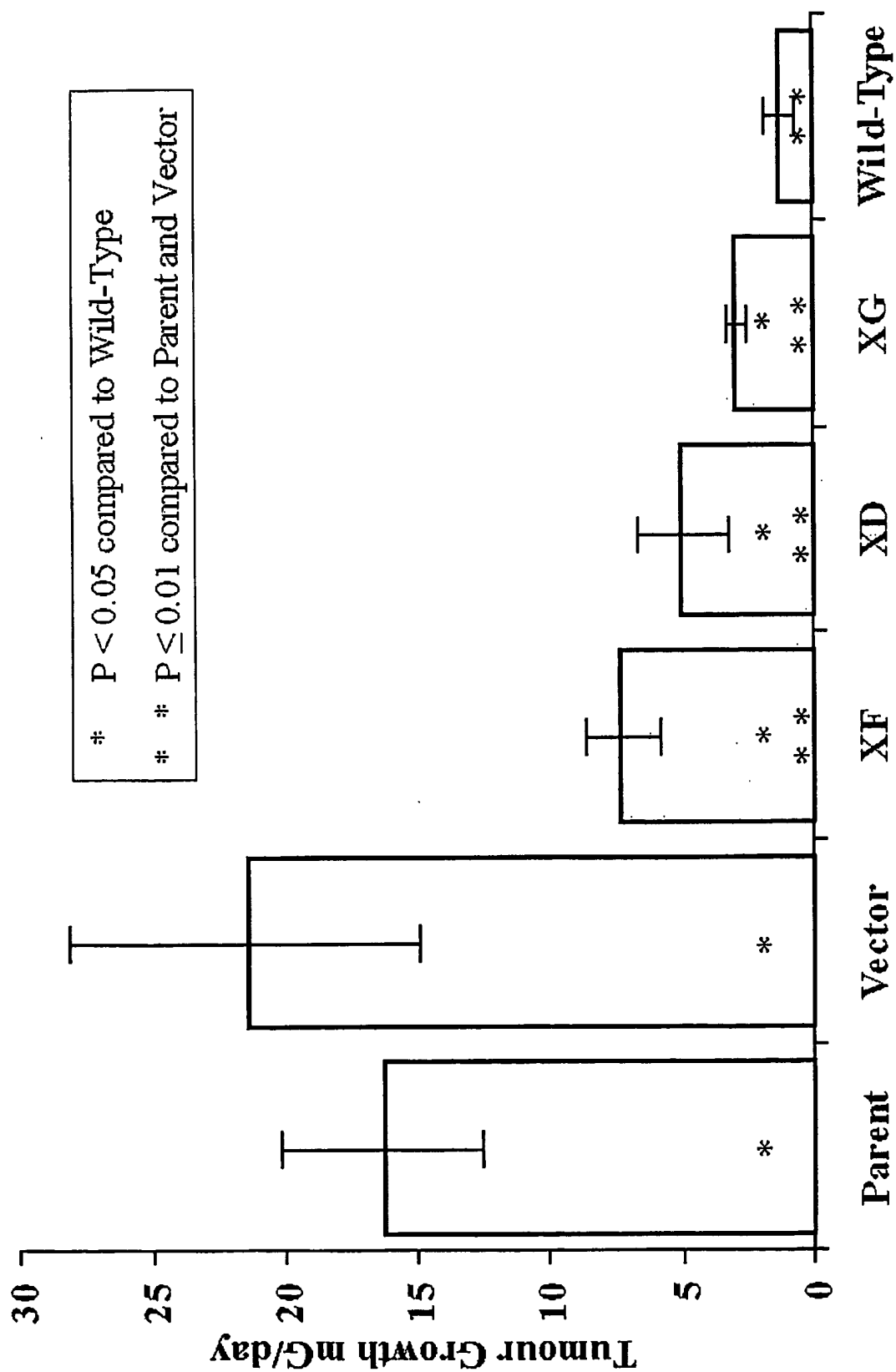
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	E E S S	E E	
	G G G G	R	
	G G G G	E	
1804	R R R S	R K	W
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	S S V	S S S S	
	G G S	S G G G	
	R R N	C R R R	
	R R N	C R R R	
	D D D D D D D D		
	S S O T S S Q Q		
	L L L L L L L L		
	- - T K T - I V -		
1815	L L L V L	V -	P F
	H H H D H Q R R		
	G G G G G A - A		
	A A G R A T A M		
	V V R K	S R R R	
	V V L L L L L L		
	D D D E D V D D		
	L L L F L V M M		
1802	T T E Q A L S D D	A	
	R C N T H D V A		
	P S V - V P A A		
	D D S R S R R K		
1798	P P P P P P P P	S	
	R R K R R W R R		
	Q Q M Q Q C Q Q		
1795	T A S S S T S S	A	
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	V V T T P P Q V V		
	G G N E G G N G		

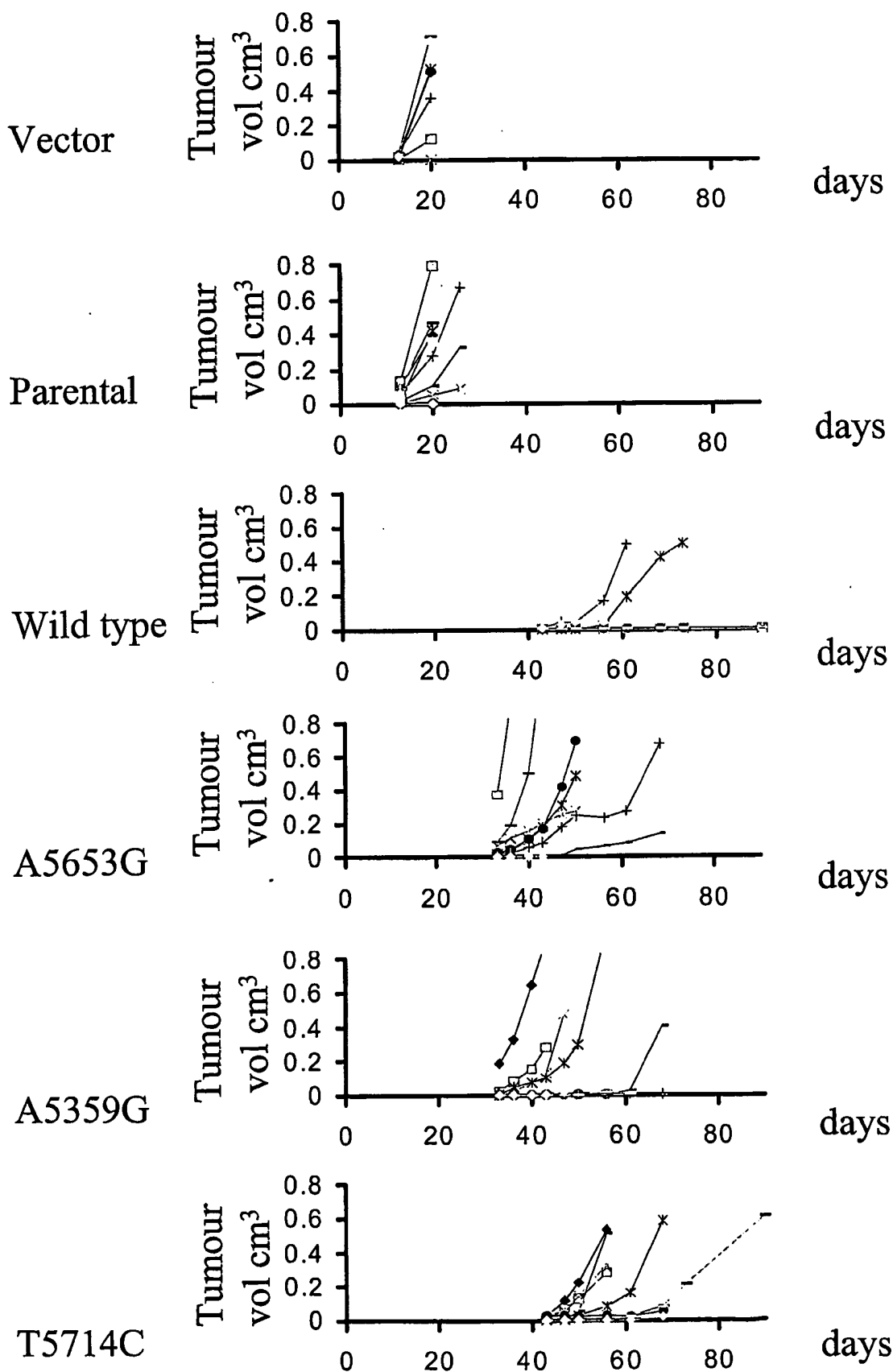
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Dm plexB
Ce plx-2
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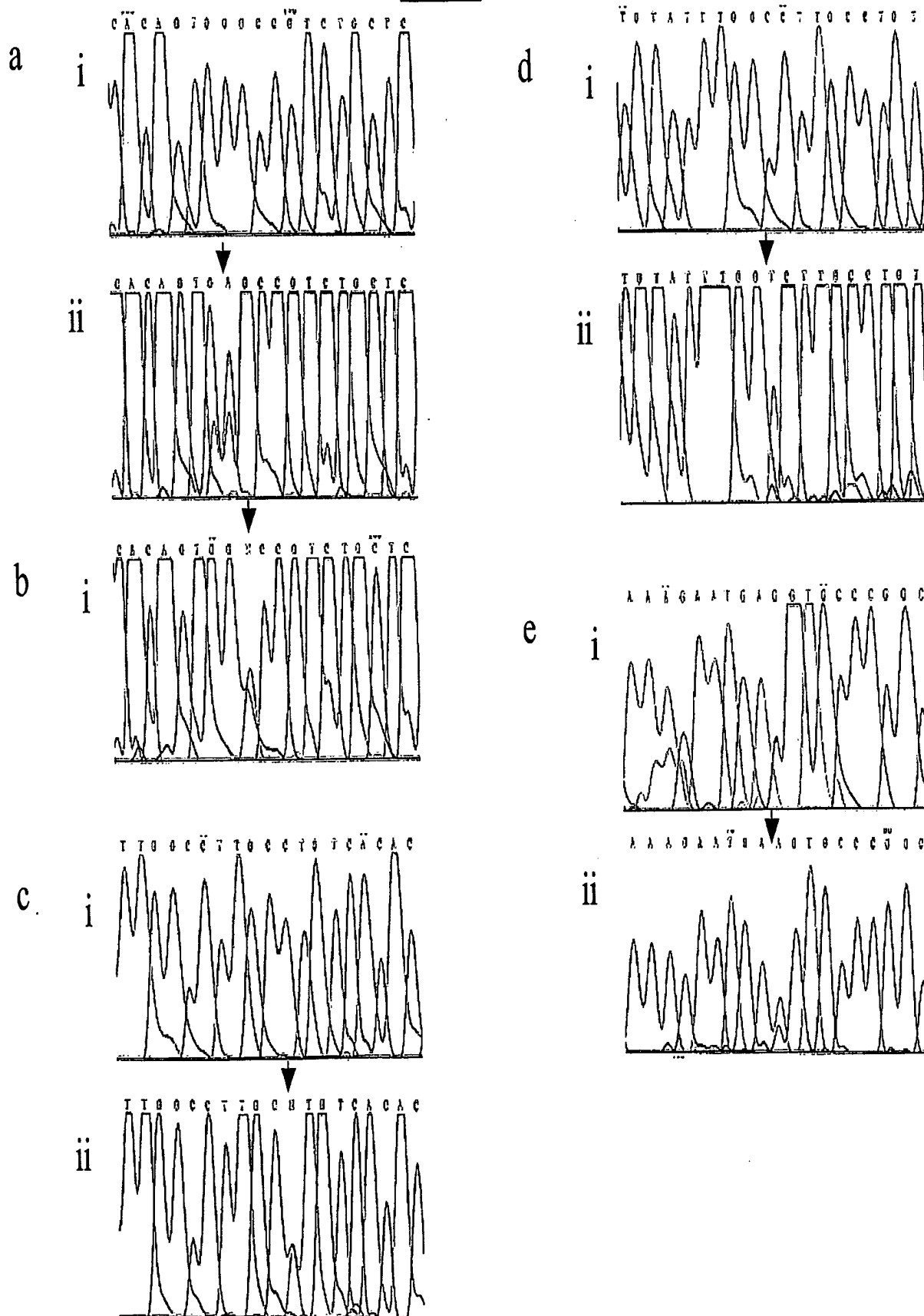
Figure 5

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Figure 6



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Figure 7

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Figure 8

INTERNATIONAL SEARCH REPORT

International Application No.

T/GB 03/05223

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/31209 A (BUBENDORF LUKAS ;MOUSSES SPYRO (US); GOVERNMENT OF UNITED STATES O) 18 April 2002 (2002-04-18) plexin 5 is plexinB1 page 72; claims 52-58; table 1	25, 36-39, 43-45
X	WO 01/54477 A (CAO YICHENG ;CHEN RUI HONG (US); HYSEQ INC (US); WANG ZHIWEI (US);) 2 August 2001 (2001-08-02) SEQ ID NO:257 = Plexin B1 table 2	60
A	WO 02/059377 A (EOS BIOTECHNOLOGY INC) 1 August 2002 (2002-08-01) claims 1-24 --- -/--	1-45, 60-64, 66,67

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search

3 May 2004

Date of mailing of the international search report

26/05/2004

Name and mailing address of the ISA

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Authorized officer

Gabriels, J

INTERNATIONAL SEARCH REPORT

International Application No

/GB 03/05223

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AURANDT JENNIFER ET AL: "The semaphorin receptor plexin-B1 signals through a direct interaction with the Rho-specific nucleotide exchange factor, LARG" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 99, no. 19, 17 September 2002 (2002-09-17), pages 12085-12090, XP002278547 September 17, 2002 ISSN: 0027-8424 the whole document	1-45, 60-64, 66,67
A	HAGA HISANORI ET AL: "Gene-based SNP discovery as part of the Japanese Millennium Genome Project: Identification of 190562 genetic variations in the human genome." JOURNAL OF HUMAN GENETICS, vol. 47, no. 11, 2002, pages 605-610, XP002278549 ISSN: 1434-5161 the whole document	1-45, 60-64, 66,67
A	BUETOW K H ET AL: "RELIABLE IDENTIFICATION OF LARGE NUMBERS OF CANDIDATE SNPS FROM PUBLIC EST DATA" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 21, March 1999 (1999-03), pages 323-325, XP000995019 ISSN: 1061-4036 the whole document	1-45, 60-64, 66,67
P,X	WO 03/031930 A (KINZLER KENNETH W ; SAHA SAURABH (US); VOGELSTEIN BERT (US); BARDEL) 17 April 2003 (2003-04-17) claim 24	25, 36-39, 43-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/05223

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 52, 54-59, 65, 68-75
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
2. ☒ Claims Nos.: 46-53
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box I.2

Claims Nos.: 46-53

Present claim 46 relates to a method defined by reference to a desirable characteristic or property, namely comprising isolating and/or purifying the test compound.

The claim covers all methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for non of such methods. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, no search has been carried out for claim 46.

Present claim 47 relates to a method defined by reference to a desirable characteristic or property, namely comprising modifying the test compound to optimise its pharmaceutical properties.

The claim covers all methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for non of such methods. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, no search has been carried out for claim 47.

Present claims 48 and 49 relate to a method defined by reference to a desirable characteristic or property, namely comprising formulating said compound with a pharmaceutically acceptable excipient.

The claims cover all methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for non of such methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, no search has been carried out for claims 48 and 49.

Present claims 50-53 relate to a compound defined by reference to a desirable characteristic or property, namely being identified as a putative anti-cancer agent by the method of claim 45.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for non of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, no search has been carried out for claims 50-53.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

GB 03/05223

Form PCT/ISA/210 (patent family annex) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No

/GB 03/05223

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03031930	A	17-04-2003	WO 03031930 A2	17-04-2003



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